STANDARDIZATION AND PHARMACOLOGICAL SCREENING OF *POONAGA PARPAM*

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TABLE CONTENTS

FIGURE CONTENTS

CHART CONTENTS

ABBREVIATIONS

ANOVA	Analysis Of Variance
EDAX	Energy Dispersive X-Ray Analysis
FTIR	Fourier Transform Infrared Spectroscopy
IAEC	Institutional Animal Ethical Committee
ICMR	Indian Council of Medical Research
ICP-OES	Inductively Coupled Plasma Optic Emission Spectroscopy
PNP	*Poonaga Parpam*
SEM	Scanning Electron Microscope
SOP	Standard Operative Procedure
TGA	Thermo Gravimetric Analysis or Thermal Gravimetric Analysis
WHO	World Health Organization
XRD	X-Ray Diffractometer

1. INTRODUCTION

All over the world traditional systems of medicine have become significantly more popular because of the curative property, less toxic and minimal side effects. Siddha medicine is a unique one as it is not only a curative but also preventive and to achieve the healthy body and mind. Siddha medicines revitalize and rejuvenate the body. It is well known that all the eyes of the world are turning to the natural medicine, especially indigenous system of medicine to find out a more acceptable drug for incurable diseases.

All Indian systems of medicine use plants, minerals and animal products as main ingredient to cure various ailments. It is more widely used for the human ailments from time immemorial. The mode of preparation and plant used in traditional medicine varies from place to place. Siddha system of medicine is the oldest holistic management system with meticulously documented medicines and being practiced by a large population in south India.

Siddhars believed that there is a connection between the external world and the internal world, which is universe as macrocosm and human as microcosm. Any change in the external world brings changes in man.[1] It is quoted by the great Siddhar Sattamuni as

அண்டத்தில் உள்ளதே பிண்டம்

பிண்டத்தில் உள்ளதே அண்டம்

அண்டமும் பிண்டமும் ஒன்றே

அறிந்துதான் பார்க்கும் போதே

-சட்டமுனி நாதர்

The volumes of literatures in Siddha system of medicine describes about 4448 diseases. Apart from the usage of herbs, it could assert that the Siddhars were the pioneers in the use of minerals, metals and animals in the treatment of diseases.

Saint *Thirumular,* the one among eighteen siddhars define medicine as follows

மறுப்பது உடல் நோய் மருந்தென லாகும்

மறுப்பது உளநோய் மருந்தெனச் சாலும்

மறுப்பது இனிநோய் வாரா திருக்க

மறுப்பது சாவை மருந்தென லாமே.

According to *Thirumular*, the above lines can be explained as medicine means one that ensures physiotherapy, psychotherapy, prevention and prevention against untimely mortality.[2]

Respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) are a serious health problem, which are increasing rapidly worldwide. The current therapy has its own shortcomings and notable adverse effects.

'Asthma' is a Greek word which means 'breathless' or 'to breathe with open mouth'. Bronchial asthma is an important allergic disorder and it is defined as 'a chronic inflammatory disorder of the airways associated with increased airway hyper-responsiveness, recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night/early morning. It can be triggered by various factors like allergens, drugs, respiratory infection, dust, cold air, exercise, emotions, occupational stimuli, chemicals, histamine and also hereditary. The incidence of bronchial asthma is increasing nowadays. Prevalence of asthma is between 100 and 150 million people around the globe and India has an estimated 15-20 million asthmatics. The prevalence of current asthma was 11.9% in children. Allergies in Childhood (ISAAC) have provided data on asthma prevalence in 6-7 and 13-14 year old Indian children[3].The above findings indicate that the burden of bronchial asthma in Indian children is higher. In chronic asthmatics, the aim is to prevent bronchospasm.

The World Health Organization recognizes Asthma is a disease of major public health importance and place a unique role in the co-ordination of international efforts against the disease. The drugs like Bronchodilators, Anti inflammatory agents, Mast cell stabilizers, LT receptor antagonists are used for Bronchial asthma all over the world. Both prevention of inflammatory response and bronchial hyperactivity are

important for the long term control of asthma. Despite the availability of a wide range of Anti-asthmatic drugs, the relief offered by them is mainly symptomatic and short lived with more or less side effects.

In Siddha system the symptoms of Bronchial asthma can be correlated with the symptoms of *Swasakasam* as quoted by Yugi muni [4]. In other system of medicine, three different drugs such as bronchodilators, anti inflammatory and sometimes anti pyretic were used for the management of bronchial asthma. But in our siddha system, single formulation can be used for the treatment of bronchial asthma which has all these properties. Many herbal, herbo-mineral and animal origin formulations which has bronchodilator, mast cell stabilizer, and anti – inflammatory activity are used for the treatment of bronchial asthma (Swasa kaasam).

Poonaga parpam is an animal origin siddha formulation which is quoted in the text "*Sikicha Rathina Deepam"* that is indicated for bronchial asthma[5]. It is a fine ash obtained through incineration. *Poonagam* (earthworm) and *Aaduthinnapaalai* (*Aristolochia bracteata*) are used as an ingredient for the preparation of *Poonaga parpam* . It is prepared through the special oxidation procedure involving purified form of earthworm processed under herbal juice. This trial drug has been used for the management of bronchial asthma by traditional healers and siddha physicians and there is also scientific data for its safety.

Siddha system which has got a hoary of antiquity is based on five elemental Theory (*Pancha Pootha Theory*). The therapeutic potency of any drug were designed depending on the following parameters namely

- *Suvai*
- *Gunam*
- *Veeriyam*
- *Vibhaham*[6]

In siddha system of medicine there is an interrelation between *veeriyam* and treatment. All the raw drugs that is *Poonagam* (earthworm) and *Aaduthinnapaalai* (*Aristolochia bracteata*) and the finished medicine *Poonaga parpam* , a nano sized formulation possesses *veppa veeriyam* which is mainly related to the treatment of Bronchial asthma.

Standardization of Siddha preparations is an important task in establishing the safety and efficacy of the drug .The World Health Organization (WHO) guidelines on evaluating the physicochemical properties and other parameters for the identification of AYUSH formulations which will offer a great value in global market. Standardization ensures the quality of medicines and gives authenticity to the medicines prepared by the manufacturers, satisfaction of the prescribing physicians and relief to the consumers. Characterization of Siddha formulation renders wide range of information in predicting the nature and structure of phyto constituents which renders the actual therapeutic efficacy of the formulation.

So the Researcher chosen this formulation for the standardization by analyzing the physicochemical properties, chemical analysis, elemental analysis and screening the pharmacological activities of Bronchodilator, Anti inflammatory and Anti pyretic activities on animal model.

2. AIM AND OBJECTIVES

Aim

To Standardize and evaluate the pharmacological profile of the test drug *"Poonaga Parpam"* in a animal model.

Objectives

The following methodology was adopted to Standardize and evaluate the pharmacological activity of the test drug:

- Collection of various information relevant to the study which include Siddha and Modern aspect of drug, pharmacological and pharmaceutical review.
- Identification of raw drugs in *Poonaga Parpam.*
- Preparation of *Poonaga Parpam* as per classical Siddha literature.
- **Standardization of test drug**
    - To standardize the drug through physicochemical analysis.
    - To analyze the drug chemically for detection of acid, basic radicals and heavy metals.
    - To estimate the percentage of elements, functional groups and particle size through Instrumental analysis of the trial drug.
- **To determine the following pharmacological activities**
    - Bronchodilator activity by Histamine induced Bronchoconstriction in Guinea pig model.
    - Anti-inflammatory activity by Carrageenan-induced rat paw edema in Wistar albino rat model.
    - Antipyretic activity by Brewer's Yeast induced method in Wistar albino rat model.

3. MATERIALS AND METHODS

Standard Operative Procedure for preparation of "*Poonaga parpam*"[5]

The test drug *Poonaga parpam*, mentioned in Siddha text "*Sikicha Rathina Deepam"*, 2007 edition, pg no 232, has been used for *Kodiya Kaasam* (Asthma), *Mega Suram* (Syphilitic Fever), *Thagam* (Thirst).

The ingredients of this formulation are

1. Purified *Poonagam* (Earthworm)	1 *Veesai* (1400gm)
2. Juice of *Aduthinna paalai* (*Aristolochia bracteata*)	1 litre
3. Butter milk	3 litres

Collection of Raw Drugs:

The *poonagam* (Earthworm) and *Aduthinna paalai (Aristolochia bracteata*) was collected from in and around Thanjavur district, Tamilnadu. All the ingredients were purified and the medicine was prepared in the *Gunapadam* laboratory of National Institute of Siddha.

Identification and Authentication of the drug:

The *poonagam* (Earthworm) was identified and authenticated by competent authority of Gunapadam Department, National Institute of Siddha, Tambaram sanatorium, Chennai.

Aduthinna paalai (*Aristolochia bracteata*) was identified and authenticated by Botanist, Department of Gunapadam, National Institute of Siddha, Tambaram sanatorium, Chennai.

Purification (*Suddhi*) [5,7,8]:

The earth is considered to be a source of valuable drugs. Earthworms not only enriched with pharmaceutically useful compounds but also contain certain toxic materials. One of the most important tasks is removal of these toxic substances, which is called purification (*Suddhi*) of raw materials by Siddhars. Otherwise it may results in toxicity. *Suddhi* contributes the following changes in the raw drug:

- Reduction in particle size
- Conjugation of trace elements
- Elimination of unwanted elements
- Formation of desirable compounds

Poonagam

Poonagam was soaked in buttermilk. When it repels the sand it was taken out and dried, then it was grounded.

Aduthinna paalai

Aduthinna paalai was washed in the running tap water to remove the soil and impurities.

Preparation of *Poonaga parpam*:

Procedure:

Purified *Poonagam* was grounded well using mortar and pestle and juice of *Aduthinna Paalai* (*Aristolochia bracteolata*) was added little by little to it for one day and made into pellet and dried. The pellet was then placed in between two earthen saucers and it was covered by mud sealed cloth. Then it was subjected into *pudam* by using 100 cow dung cakes. The above mentioned procedure was repeated for 9 times and finally the *parpam* was powdered well and stored in an air tight container.

Labelling:

Name of the preparation	:	*Poonaga parpam*
Dose	:	½ - 1*Kundri*(65-130 mg) Twice daily
Adjuvant/Vehicle	:	After food with Honey
Route of administration	:	Oral
Indications	:	*Kodiya Kaasam* (Asthma)
		Mega Suram (Syphilitic Fever)
Date of expiry	:	100 years from the date of manufacture

INGREDIENTS OF POONAGA PARPAM

Fig.No.1 *Poonagam* (Earthworms)

Fig.No.2 *Aaduthinna paalai (Aristolochia bracteata)*

Fig.No.3 Mud Sealed Earthen Saucer

Fig.No.4.1

Fig No.4.2

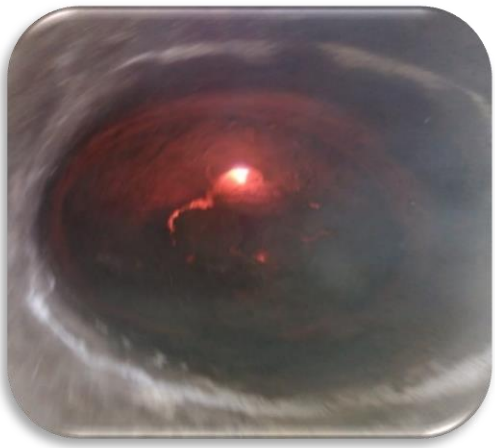


Fig.No.4.3

Fig.No.4.4

Fig.No.4 *Pudam* process

Fig.No.5 ***Poonaga parpam***

4. REVIEW OF LITERATURE

4.1 GUNAPADAM REVIEW

Poonagam (Earthworm)[9]

It is mostly available in slough. In Siddha aspect, there are two varieties of earthworms i.e.,reddish and pale red coloured.

Reddish earthworms have great medicinal values because of its more copper content.

Synonym

- *Naankuzh Pulu*
- *Naaku Poochi*
- *Kandapadham*
- *Boomiver*

General characteristics of *Poonagam*

மாதவறு செய்வறட்சி மாறுமடங் காச்சந்நி

பாதவறு நோயோடு பாறுமடல்- வாதவாறு

குண்டபதமீளு மோக்காள மையமும்போங்

கண்ட பதமென்னுங் கால்

- *Pathartha Guna Sinthamani*

Action

It is mainly used to treat severe thirst, crural paralysis (paralysis of the thighs characterized by loss of motion and sensation arising from the deranged condition of wind humor in the lower part of the body), vomiting and phlegmatic diseases.

Purification

The earthworms can be taken out after soaking it in milk or buttermilk. If the lime water is poured on the earthworms, they will die.

Medicinal uses

- ✓ The purified and dried earthworms are made into *chooranam* form. 12.6 gm of the *chooranam* along with grape juice used for the treatment of retention of urine and calculus in the urinary tract.
- ✓ When it is given in non – vegetarian soup, it improves sperm production.
- ✓ When applied topically with almond ghee, it will reduce hernia.
- ✓ When it is given internally with boiled gingelly oil, it cures chronic cough and throat pain.
- ✓ When it is ground raw and applied topically the nerves which are cut will regenerate. When ground with black stone and applied topically, it will correct the dislocation of joints. It will also dissolve the blood clot caused by injury.
- ✓ The earthworm is fried in gingelly oil along with equal quantity of dried leech. It is then applied over the penis for improving the sexual vigour.
- ✓ When the earthworm is used for medicinal purposes, ghee, milk, and meat should be taken in excessive quantities.

Siddha formulations using *Poonagam* as ingredient

- *Poonaga chenduram*
- *Poonaga Karukku*
- *Poonaga Karukku Kudineer*
- *Seenthil Chooranam*
- *Poonaga Chooranam*
- *Thanga Chenduram*
- *Varmaani Kuligai*
- *Nava Paasana Poonaga Ennai*
- *18 Kirigaikkum Kulambu*

AADUTHINNA PAALAI – Aristolochia bracteata[10,11]

Synonym

- *Aadu theenda paalai*
- *Aadu thodappalai*
- *Pangampaalai*

Parts Used

Leaves, seed, root or whole plant

Organoleptic Characters

Taste : Bitter

Character : Heat

Division : Acrid

Action

- Anthelmintic
- Emmenogogue
- Stimulant
- Tonic
- Purgative
- Alterative
- Anti periodic

General characteristics of *Aaduthinnapaalai*

கிரந்திகரப் பன்வெக்கை கேசநலி மாந்தை

யரந்தை வினையை யறுக்கும்- துறந்து

பிரியொணா நோய்களையும் பின்முன்பாராமல்

மறியுணா முீலியடை வாய் *-Theraiyar Venba*

ஆடுதொடாப் பாளைக் ககக்கருமி வன்சிலந்தி

நீடுகருங் குட்டம் நிறைகரப்பான்- ஆடிடச்செய்

எண்பது வாய்வும் இகல்குட்ட முந்தீரும்

திண்பெறுநற் றாதுவுமாஞ் செப்பு.

-Agathiyar Gunavakadam

It cures skin eruptions, glandular swelling, hair loss, congenital heat in children. It also Cures intestinal worms infestations, ringworms, 80 types of vadha diseases.

Medicinal Uses:

- The decoction of the leaves is given for above mentioned ailments.
- The powdered seed along with castor oil can be used to cure stomach ache, amenorrhoea, intermittent fever, labour pain. It also cures intestinal worm infestations.
- The whole plant along with gingelly oil can be used externally to cure ringworm, eczema.
- The powdered root is used to cure snake bites and other poisonous bites.

Siddha formulations using *Aaduthinnapaalai* as ingredient

- *Aadutheendapaalai Ennai*
- *Thuvar Ennai*
- *Mooku Noi Thailam*
- *Aadutheendapaalai Maathirai*
- *Milagennai*

4.2 ZOOLOGICAL REVIEW

EARTHWORMS

Earthworms are macroscopic clitellate oligochaete annelids that live in soil. It is the common name for certain terrestrial members of the class Oligochaeta, especially forms belonging to the family Lumbricidae.

Vernacular names

- Tamil : *Poonagam/ Manpuzhu*
- English : Earthworm / Red worm/night walker
- Sanskrit : *Bhūjantuḥ*
- Telugu : *Vānapāmu*
- Kannada : *Mannuhulu*
- Malayalam : *Maṇṇira*
- Hindi : *Kenchua*
- Gujarathi : *Aḷasiyā*
- Bengali : *Kēm̆cō*
- Urdu : *Kechwa*

Scientific Classification

Kingdom	:	Animalia
Phylum	:	Annelida
Class	:	Oligochaeta
Subclass	:	Oligochaeta
Order	:	Megadrilacea
Suborder	:	Lumbricina + Moniligastrida

Characters & Description[12]

- There are 2,200 earthworm species, found all over the world except in arid and arctic regions and ranging in size from 1 in. (2.5 cm) to the 11-ft (330-cm) giant worms of the tropics. Some earthworms are pallid in color, many are reddish brown to purple, and one Philippino species is bright blue.

- Earthworms burrow in the ground, swallowing soil from which the organic material is extracted and ground up in the gizzard and depositing the residue as castings outside the burrow. They come to the surface only on cloudy days and at night (hence the name night crawlers) unless they are flooded out by heavy rainfalls. In cold and dry weather they retreat into their burrows and remain dormant.
- The segments of the earthworm, visible externally as rings, are separated by internal partitions. On each segment are four pairs of bristles, or setae, with which the worm anchors it selves to the walls of the burrow, drawing itself forward by rhythmic muscular contractions. There is a nerve cord, with ganglia in each segment and an enlarged cerebral ganglion (a primitive brain) at the anterior end. Although they have no prominent sense organs, earthworms are sensitive to light, touch, vibration, and chemicals.
- The circulatory system is enclosed in vessels; the blood (which contains haemoglobin) is pumped by muscular contractions of five linearly arranged hearts.
- Earthworms are hermaphroditic, but they cross-fertilize. Two worms exchange sperm cells during copulation; fertilization occurs after the worm's own eggs and the received sperm are encased in a tough sheath secreted by the clitellum, a conspicuous band of tissue near the anterior end. The sheath slips over the worm's head and is deposited underground, where it serves as a cocoon for the developing young. There is no larval stage; the young hatch as miniature adults.
- The common American and European earthworm, *Lumbricus terrestris*, up to 10 in. (25 cm) long, with about 150 segments, is used for laboratory dissection and study.
- Earthworms are also used as live bait and are eaten by some peoples—such as the Maoris, who consider certain species delicacies.
- The earthworm's greatest service, however, of immense importance to agriculture, is aerating and mixing the soil. Earthworm castings bring to the surface from 7 to 18 tons of soil per acre annually.
- Earthworms(*L.rubellus*) plays an important role in human life because of its high nutrient content, especially protein (64-76%).

Ecology[13]

Earthworms are classified into three main Eco physiological categories:

(1) Leaf litter- or compost-dwelling worms that are non-burrowing, live at soil-litter interface and eat decomposing OM (called Epigeic) e.g. *Eisenia fetida*;

(2) Topsoil or subsoil-dwelling worms that feed (on soil), burrow and cast within soil, creating horizontal burrows in upper 10–30 cm of soil (called Endogeics); and

(3) Worms that construct permanent deep vertical burrows which they use to visit the surface to obtain plant material for food, such as leaves (called Anecic which means "reaching up"), e.g. *Lumbricus terrestris*.

Earthworms form the base of many food chains. They are preyed upon by many species of birds (e.g. starlings, thrushes, gulls, crows, European robins and American robins), snakes, mammals (e.g. bears, foxes, hedgehogs, pigs, moles) and invertebrates (e.g. ground beetles and other beetles, snails, slugs). Earthworms have many internal parasites, including protozoa, platyhelminthes, and nematodes; they can be found in the worms' blood, seminal vesicles, coelom, or intestine, or in their cocoons.

Action

- Antioxidant
- Antiulcer
- Hepato-protective
- Anticancer
- Anti Pyretic
- Anti Inflammatory
- Wound healing
- Fibrinolytic/fibrinogenolytic,
- Thrombolytic and anti-coagulative Activity.

Earthworms have been used as a traditional medicine in China, Japan and the other Far East countries for thousands of years. Extraction and purification of the

bioactive material contained in the protein earthworms have been conducted in various countries.

❖ **Nutritional Profile of Earthworm Powder of *Lampito mauritii.***

➢ Carbohydrate	4.1 %
➢ Protein	31.7 %
➢ Nitrogen	1.83%
➢ Phosphorus	0.38%
➢ Potassium	0.53%
➢ Iron	241.1 (ppm)
➢ Magnesium	0.213(ppm)
➢ Manganese	17.2 (ppm)
➢ Zinc	32.34 (ppm)
➢ Copper	4.50(ppm)
➢ Calcium	0.280(ppm)

Earthworms have long been utilized as a form of nutrition. Scientists found that earthworms contain 78-79 g/L free amino acids and they contain a high concentration of important vitamins and minerals such as iron and calcium. They investigated the diet of Amerindians of the Amazonas in Venezuela. They found that these peoples used leaf and litter-feeding invertebrates as a primary source of proteins, fats, and essential vitamins.

The Maori, or indigenous Polynesian population of New Zealand as well as various Australian aboriginal populations have been noted to use earthworms as food sources.

Influence of Environmental Factors on Survival and Growth of Earthworms[14]

A. Temperature

Earthworms have fairly complex responses to changes in temperature. They studied the potential of several earthworm species to grow in sewage sludge, and they concluded that all these species have a range of preferred temperatures for growth, ranging between 15°C (59°F) and 25°C (77°F). In extreme temperature conditions earthworms tend to hibernate and migrate to deeper layers of the windrow for protection.

B. Moisture Content

There are strong relationships between the moisture content of organic wastes and the growth rate of earthworms. In vermicomposting systems, the optimum range of moisture contents for most species has been reported to be between 50% and 90%.

C. pH

Most epigeic earthworms are relatively tolerant to pH and can tolerate pH levels of 5–9, but when given a choice in the pH gradient, they move toward the more acid material, with a pH preference of 5.0.

D. Aeration

Earthworms lack specialised respiratory organs, and oxygen and carbon dioxide diffuse through their body wall. Thus, earthworms are very sensitive to anaerobic conditions.

E. Ammonia and Salts

Earthworms are very sensitive to ammonia and cannot survive in organic wastes containing high levels of this Cation (e.g., fresh poultry litter). They also die in wastes with large quantities of inorganic salts.

Characteristic composition in earthworm [15]

- Earthworms usually contain some characteristic compositions of lumbrofebrine, terrestrolumbrlysin, lumbritin, hypoxanthine and other purines, pyrimidines, choline and guanidine.
- The fat of earthworm is composed of octade acids, palmitic acids, high-chain unsaturated fatty acids, linear and odd carbon fatty acids, branched fatty acids, phosphatide.
- The yellow chloragenous cells and organs of *Lumbricus terrestris* contain rich amounts of carbohydrates, lipids, protein, pigments and some alkaline amino acids.
- The neutral fat consists mainly of laurel acid, oleate, myristic acid and decanoic acid. The fatty acids of glucolipids are decanoic acid and some short chain fatty

acids. The fatty acids of phosphatide are oleate, decanoic acid, linoleate and behenic acids.

- A P-peptide substance exists in the gut wall of *Lumbricus terrestris*. Some active enzymes occur in the yellow chloragenous cells and organs of *Lumbricus terrestris* in high concentrations, including catalase, peroxidase, dismutase, β-D-glucosyl enzyme, alkaline phosphatase, esterase, S-amino-γ-ketoglutaric dehydrogenase and porphyrin synthetase.
- Scientists from Japan, China and Korea found and separated enzymes from the earthworm gut and body fluids, which can dissolve fibrin. These enzymes have been developed as innovative medicines to treat cerebral thrombosis and myocardial infarction.

LUMBROKINASE, an active component

Lumbrokinase, an organic compound derived from earthworms has been shown to offer a wide range of health benefits. Lumbrokinase is now used as a dietary supplement. The Lumbrokinase possesses both anti-thrombolytic and Anti-platelet activity.

Lumbrokinase (LK) is a group of six novel proteolytic enzymes derived from the earthworm *Lumbricus rubellas*. The enzymes:

• Have potent fibrin-dissolving properties

• Decrease fibrinogen

• Lower blood viscosity

• And markedly reduce platelet aggregation.

- Recent research suggests that LK may be effective in the treatment and prevention of ischemic heart disease, as well as myocardial infarction, thrombosis of the central vein of the retina, embolism of peripheral veins, and pulmonary embolisms.
- One remarkable property of lumbrokinase is that, unlike the medications streptokinase and urokinase, it is only active in the presence of fibrin. Though it dissolves fibrinogen and fibrin very specifically, it hardly hydrolyzes other important blood proteins such as plasminogen or albumin. It has the profound advantage of not causing hemorrhage due to excessive fibrinolysis.

- Toxicological experiments have found no negative effects of Lumbrokinase on nervous, cardiovascular, respiratory and blood systems of rats, rabbits and dogs.

Therapeutic uses

- ✓ An enzyme complex isolated from earthworms increases the levels of tissue plasminogen activator (t-PA, a protein involved in breakdown of blood clots) and consequently shown fibrinolytic activity – without harmful side effects.
- ✓ In a study in 2000 the complex was found to be beneficial for ischemic (clot-caused) stroke, without increasing the risk of excessive bleeding as other anticoagulants can.
- ✓ Using spectrofluorimeter and flow cytometry, a study found that this complex has Anti-platelet activity, Anti-thrombotic activity and anti-apoptotic activity.
- ✓ According to the most famous ancient Chinese *materia medica*, earthworms could treat hemiplegia, fever, and blood clots.
- ✓ Earthworms can kill bacteria and lyse foreign cells; their body fluid contains leukocytes that are as varied as those of many vertebrates.

4.3 BOTANICAL REVIEW

***Aristolochia bracteata* Retz.**

Aristolochia bracteata is a large plant genus with over 500 species, belongs to the family *Aristolochiaceae.* It is an important medicinal herb and it is an origin of Indian subcontinent and has become naturalized in the tropical and sub-tropical areas around the world.

Common names

Worm-killer, Indian Birthwort, Bract eated Birthwort

Vernacular names

Sanskrit	:	*Ajaspurisaha, Aulosa, Hukka-Bel, Kalipaad*
Hindi	:	*Gandan, Aulosa, Hukka-Bel, Kalipaad, Kiramar*
Tamil	:	*Aadutheenda Paalai*
Kannada	:	*Sanajali-Hullu, Aadumuttada Gida, Kalaguraki*
Telugu	:	*Kadapara, Gadide- Gade-Para-Aku*
Malayalam	:	*Atu-Tintap-Pala*
Marathi	:	*Gandhaari, Gindhaan, Keedamaari*
Bengali	:	*Kiramar*
Gujarathi	:	*Kidaamaari*
Punjabi	:	*Kitamar*

Scientific classification

Kingdom : Plantae

Phylum : Tracheophyta

Class : Magnoliopsida

Order : Piperales

Family : Aristolochiaceae

Subfamily : Aristolochioideae

Genus : *Aristolochia*

Species : A. *bracteolate*

Binomial name : *Aristolochia bracteata*

Distribution[16]

This species is globally distributed in Tropical Africa, Arabia, Sri Lanka, Pakistan and India. Within India, it is found in northern and central India from Haryana to West Bengal and southwards to Tamil Nadu and Kerala. It is common in dry areas, particularly on black cotton soil, usually growing as a weed.

Ecology

Aristolochia bracteata grows in subsaharan regions from Mali to Somalia through to the Arabian peninsula and India. The plant grows at elevations of 50-740m above sea level and and can be found on the banks of rivers, bushland, desert grasslands. It grows in sandy or lava soils.

Cultivation [17]

Aristolochi bracteata is usually gathered from the wild.

Description[18]

a) Macroscopic:

Aristolochia bracteata is a herbaceous perennial medicinal plant with cordate leaves and dark purple colour tubular flowers belonging to the family Aristolochiaceae. The climbers reach heights of 10 to 50 centimetres.

Leaves

- The simple leaves are alternate and cordate, membranous, entire and petiolate, growing on leaf stalks.
- There are no stipules.

Flowers

- The flowers grow in the leaf axils.
- They are inflated and globose at the base, continuing as a long perianth tube, ending in a tongue-shaped, brightly colored lobe. There is no corolla. The calyx is one to three whorled, and three to six toothed.
- The sepals are united (gamosepalous). There are six to 40 stamens in one whorl. They are united with the style, forming a gynostemium.
- The ovary is inferior and is four to six locular. These flowers have a specialized pollination mechanism. The plants are aromatic and their strong scent attracts insects. The inner part of the perianth tube is covered with hairs, acting as a fly-trap. These hairs then wither to release the fly, covered with pollen.

Fruit

- The fruit is dehiscent capsule with many endospermic seeds. Capsule 2-3 cm long, 12-ribbed, glabrous. Seeds may, 5-7 mm long, flat and dark coloured.

b) Microsocopic:

Leaf-

Petiole

- TS almost angular in outline, with one depression on the upper and two depressions on the lower surface; epidermis single layered followed by 3 or 4 rows of collenchyma; below the ridges about 4 or 5 layers of chlorenchyma present; vascular bundles five in number arranged in a shallow arc; ground tissue parenchymatous.

Midrib

- Midrib shows a slightly convex outline adaxially, and almost circular abaxially; epidermal cells single layered; the upper and lower sub-epidermal region composed of 2 to 4 layers of collenchyma; a single vascular strand present; ground tissue is made up of parenchyma cells; unicellular epidermal hairs present on abaxial epidermis.

Lamina

- TS shows dorsiventral structure; epidermis single layered, composed of rectangular cells; trichome occasional on upper surface, simple and unicellular; palisade single layer; spongy tissue composed of loosely packed circular to oval cells; vascular strands present; stomata anomocytic, present on both epidermis; in surface view, adaxial epidermal cells straight walled, but abaxial cells rather wavy; stomatal number 6 to 9 /mm^2 for adaxial epidermis and 23 to 27 / mm^2 for abaxial epidermis ; stomatal index for adaxial epidermis 6 to 12 and for abaxial epidermis 16 to 24; palisade ratio 5 or 6; vein islet number 8 to 12.

Powder

- Greyish green, shows the presence of palisade cells, fragments of epidermis with straight or slightly wavy walls and anomocytic stomata, parenchyma and collenchyma cells seen, vessels with helical, mostly scalariform and occasionally pitted thickenings on walls observed.

Agronomic characters[19]

It can be propagated by seeds which germinate in about two weeks. Seeds can be collected by bagging the fruits. The seeds are sown in June in well mannered beds. Seedlings are transplanted after six weeks and trained on to bamboo platforms. The vines flower in September and fruit during February - March. They are allowed to grow for two years to yield roots of marketable size. The yield of roots is estimated at 4500-5600 kg/ha from two year old vines. The roots are collected in the autumn. The adhering earth is removed by minimum washing. The roots are dried in the sun or by gentle heat.

Plant part used

Whole plant, Leaves, Seeds, Roots.

IDENTITY, PURITY AND STRENGTH –

Foreign matter - Not more than 2 per cent

Total ash - Not more than 10 per cent

Acid-insoluble ash - Not more than 1.3 per cent
Alcohol-soluble extractive - Not less than 12.8 per cent
Water-soluble extractive - Not less than 25.5 per cent
Fixed oil - Not less than 5.3 per cent

Phytochemical constituents

- Methanolic extract of plant parts of A. bracteata was the source of physiological active compounds.
- The secondary metabolites from Aristolochia species cover 16 major groups classified by their chemical structures, including aristolochic acids and esters, aristolactams, aporphines, protoberberines, isoquinolines, benzylisoquinolines, amides, lignans, biphenyl ethers, coumarins, tetralones, terpenoids, benzenoids, magnoflorine; *N*-acetylnornuciferine; aristolactam; β-sitosterol and ceryl alcohol.
- The Phytochemical analysis of this plant has revealed the presence of alkaloids, triterpenoids, steroids, sterols, flavonoids, tannins, phenolic compounds and cardiac glycosides.
- Considerable amounts of reducing sugars in free form are present in the roots. The chief active principle of the drug is aristolochic acid, though aristolic and p-coumaric acids also appear to contribute to the activities of the drug.
- Aristolochic acid is 8-methoxy-3; 4-methylenedioxy – 10 – nitrophenanthrene – 1 –carboxylic acid. It is intensely bitter and is optically inactive.

Properties and medicinal uses [20]

- ✓ Decoction of the whole plant is given in fever, worms infestations, skin disease and snake bite.
- ✓ The plant is used in traditional medicine as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snake bites, antimicrobial activity, anti-arthritic activity, anti-allergic activity and anti-oxidant property.

- ✓ The ethyl acetate, acetone and methanol extraction of roots showed promising antibacterial activity in Gram positive and Gram negative bacterial dish. Among them ethyl acetate extract was found to be the most effective.
- ✓ The freshly bruised leaves are mixed with castor-oil and used in Nigeria topically on pimples.
- ✓ Roots mixed with lime juice are taken for snake bite and scorpion stings in Nigeria. In East of Lake Chad the root is also applied topically to scorpion stings.
- ✓ The flowers are sometimes worn in Northern Nigeria as charm against snake-bite and scorpion-stings.
- ✓ The use of the plant as antibiotics, antimalarial and aphrodisiac has been reported by traditional healers of Southwestern Nigeria.
- ✓ The stem and the root contain the alkaloid aristolochic acid.
- ✓ The dried, powdered root has been shown to increase the contractions of the uterus during labour. It has been used as a substitute for ergot.
- ✓ The leaves and roots are used to get rid the body of Guinea worm (a parasitic infection caused by a nematode)

Indications

Whole plant :Dermatitis, allergic disorder, leprosy, jaundice, Worms, fever, Mosquito repellent , Anodyne, purgative, emmenagogue

Leaves :Anti-inflammatory dermatitis, rashes, skin disease, for scorpion sting, Antipyretic, snake bite, Antiulcer, amenorrhoea, antihelmintic, Antiplasmodial.

Seeds : Antibacterial, anti-inflammatory and analgesics

Roots : Syphilis, gonorrhoea & skin diseases, eczema

Benefits of Worm-killer

- ✓ In the Indigenous system of medicine, the plant was used as purgative, antipyretic & anti-inflammatory agents.

- ✓ The root part has antifungal and antibacterial activity and was used to treat syphilis, gonorrhoea, and skin diseases and also used during labours to increase uterine contraction.
- ✓ Its leaves are bitter and anti-helmintic, antiulcer, anti-plasmodial and are medicinally important.
- ✓ The whole plant is very bitter and has abortifacient, alterative, anthelmintic, antiperiodic, emmenagogue and purgative properties.

4.4 PHARMACEUTICAL REVIEW

Pharmaceutics is a discipline of pharmacy that deals with the process of turning a new chemical entity to be used safely and effectively by the patients. (Formulation of pure drug substance into dosage form)

Siddha pharmaceutics has very minute chemical processes in it. It has several chemical processes like purification of raw substances, grinding them with herbal juices for several days and subjecting the ground material to fire by way of *pudam* process. Medicines prepared according to the above methods undergo several chemical changes.[21]

Siddha medicines are classified into internal medicines (32) and external medicines (32). The drug taken for dissertation is in the form of *parpam.* Other names of *parpam* are *neeru or venneeru. Parpam* comes under the category of internal medicines.

Purification of the drugs included

Purification of the drugs is mainly done to remove the toxicities, impurities like soil, dust, clay present in the drugs. Also the drugs when subjected to heat like roasting or soaked in liquids undergo certain chemical reactions such as oxidation of toxic substances to non-toxic, reduction of some poisonous chemicals to non-poisonous ones, or undergo enzymatic reactions. In these ways, not only the toxicities and impurities are removed but also enhances the potency of the drugs.

Poonagam

Poonagam was soaked in buttermilk. When it repels the sand, it was taken out and dried, then it was grounded.

Leaves of *Aduthinna paalai*

- *Aduthinna paalai* was washed in the running tap water to remove the soil and impurities.
- The mature leaves, insect infested leaves are removed.

PARPAM

Concept and terminology

Parpam is also known as '*Neeru*' . The materials that go into the preparation of '*parpam*' should first be cleaned and taken through the specified processes of purification that are recommended for each component. Then they are ground with juices of leaves, distillates or extractives and then subjected to frying or calcinations or suitably heating in the manner recommended in the recipe till the product is satisfactorily calcined.

Method of preparation

The purified drugs are crushed and converted into fine powder so that they are ground in the mortar. These powders are put into the mortar, specified juice are added and ground and the mass is made into small discs and dried in sun.

If the discs are not well dried, the '*parpam*' or the calx will not attain the specified colour, specific for the particular *parpam*. So, they are thoroughly dried and spread in a shallow earthen pan and covered with an identified pan inverted over it and the edges are sealed with clay smeared cloth ribbon.

This set up is dried and then placed and burnt in kilns of suitably pre-determined size. The calcination capsules and the contents therein should be taken only when the kiln has cooled down by itself.

General precautions

When the discs are arranged in the pans, they should not be heaped up and should not be arranged in more than one layer. Only then the heat will react on the material properly. The pans should also be not dis-proportionately big when compared to the quantify of drugs and they should not be very deep. As the colour, effect and fineness of the '*parpam*' will be enhanced according to the degree of grinding; they should be ground very finely.

The kiln is constructed by making circular excavations of suitable dimensions in places with optimum aeration and the sides are lined with bricks kilns should not be constructed in places where strong winds blow.

Usually cow dung cakes are used as fuel in kilns. However, in some specified instances some barks or goat dung and other materials are recommended. In cow dung cakes, there will be an appreciable admixture of sand or mud. Depending upon the degree to which there is such admixture, the number or weight of cow dung cakes may be increase. With adequate practical experience one can determine this correctly.

Half the number of cow dung cakes is spread at the bottom of the kiln and the calcination capsules are placed over this at the center. The remaining cow dung cakes are arranged over these and are ignited all around.

Materials like sulfur and yellow orpiment which do not withstand and strong heating are hidden in specified ashes when being calcined. In such cases, the ash is spread in the pan, the discs placed over then covered with more ash after which the other pan is inverted over and sealed along the seam. The product obtained by appropriate calcinations should be very finely ground in a mortar and taken.

Properties

In general, Parpam are always white, but *Thanga parpam* (calx of gold) is light yellow in colour. They are fine in particle size and light in weight.

“நீறிருந்த படிகளெல்லாம் நிறமது வெளுப்பே யாகும்

வேறிந்த வெளுப்பை யால்லால் வேறுநிறம் பற்பமன்று

கூறிந்த தங்கம் குலம்பொய்யா நிறமு மஞ்சள்

வாறிந்தப் படியே யல்லாமல் மற்றெல்லா மருட்டுத் தாமே”

(அகத்தியர் வைத்திய காண்டம் -600)

பற்ப மகிமை

வீரத்து மிக்கவை பற்பங்களே- பரி

காரத்து மிக்கவை பற்பங்களே

பாருக்குள் மானிடர் நோய்போக – வரு

பண்டிதருக்கெல்லா மாமேகம்

வீரகடாரி – பிணிக்கொரு

பாரகுடோரி- விசைபெறு

தீரதடாரி- வினையுடு

சூரிக்குழு நேரொத்தது

மேருக்கிணை பாரப்புறம்

(தேரையர் தரு- குணப்பாடம் தாது ஜீவ வகுப்பு)

Storage

They should be kept in dry, air tight container. When properly stored, they retain their potency for 100 years.

Adjuvant

It is advised to take along with butter, ghee, honey or milk.

Dosage and Duration

Dosage is indicated according to the age of the patient. Duration is fixed, according to the disease condition as 3 days to 48 days.

Shelf life of the drugs

The shelf life of the drugs depends on the effectiveness of the preparation. The efficacy, smell, taste and appearance of the drugs gradually change as time goes on resulting in reduced potency thereby the desired effect is not attained. But some drugs appear to be good externally inspite of reduced efficacy. So they should not be considered for consumption and should be discarded. The shelf life of *parpam* is 100 years. Also the following are the analytical parameters of specifications of *parpam,*

Testing parameters for *Parpam* -AYUSH guidelines[22]

S.NO	TESTS
1	Description, Colour, Odour
2	Identification –chemical
3	Particle size mesh size — 200 – 300
4	Loss on drying at 105 °C
5	Total – ash
6	Acid – insoluble ash
7	Water soluble ash
8	Assay of element (s)
9	Siddha specifications
10	Lusterless
11	Fine enough to enter the crevices of finger
12	Floats on water
13	Smokeless
14	Tasteless
15	Irreversible

Traditional Tests for *Parpam*

- ✓ The final product should not have the glitter or shine.
- ✓ If a small quantity is pinched and rubbed between the thumb and fore–finger the particles should be so fine as to enter and reside in the furrows and depressions.
- ✓ If a pinch of *parpam* is gently put on the surface of water in a container, the material should not sink, but it should float.
- ✓ If the *parpam* is put into a crucible and heated in a blower or oven, it should not revert to its metallic state.

4.5 SCIENTIFIC REVIEW

TOXICITY STUDY

Poonagam (Earthworm)[23]

Jaganathan Anitha et.al, studied the Oral acute toxicity study of earthworm powder in wistar male rats that showed no toxicity even at the doses of 100, 200 and 300 mg/kg. There was no significant mortality and changes in body weight noticed at all the doses tested.

Poonaga parpam[24]

Acute oral toxicity study of *Poonaga parpam* (OECD -423):

In acute oral toxicity study, there were no abnormal signs developed in wistar albino rats up to the dosage levels of 2000mg/ kg/ b.wt throughout the study period. No mortality was observed in the study period.

28 Days repeated dose oral toxicity study of *Poonaga parpam* (OECD- 407)

- ✓ Toxicity signs were not observed during the dosing period of 28 days
- ✓ All animals from control and all the treated dose groups survived throughout the dosing period of 28 days.
- ✓ The result of haematological and biochemical investigation shows no significant changes except the values of triglycerides were gradually decreased in all treated groups when compared to control group which was statistically not significant.
- ✓ The histopathological study of various organs were normal in control, low dose, mid dose and high dose group.

PHARMACOLOGICAL STUDY

Broncho Dilator Activity

- ➢ ***Poonagam* (Earthworm)[25,26]**

An effective asthma-calming component from earthworms was separated early in the 1930's. This component was used in experiments with rabbit lungs and reported that the component made bronchiectasis. Hence it could be used to resist asthma caused by histamine and pilocarpine. This component was injected Intravenousously

to the body cavity of experimental animals, 50% of the animals could withstand the lethal dose of histamine.

- ***Aduthinna paalai* (*Aristolochia bracteata*)[27]**

H r Chitme et al., studied the Antiallergic activity of *Aristolochia bracteolata* Lank in animal model. It was evaluated using compound 48/80 induced anaphylaxis, dermatitis, rhinitis and pruritis, as a preclinical model for acute phase of hypersensitivity reactions. The present study implied that the chloroform extract of *Aristolochia bracteata* had potent and significant effect in toluidine diisocyanate induced rhinitis in swiss albino mice. Mast cell membrane stabilization activity was also observed in compound 48/80 induced mast cell activation. A significant reduction was observed in serum nitrate levels, rat peritoneal fluid nitrate levels and BAL nitrate levels. The extract was also found to possess significant inhibitory effect on blood histamine levels.

Anti-Inflammatory Activity

- ***Poonagam* (Earthworm)[28]**

M.Balamurugan et al., studied the anti inflammatory activity of earthworm extract by using the carrageeenan induced left hind paw edema method. The results revealed that the histamine induced acute phase rat hind paw oedema volume and turpentine induced chronic phase granuloma pouch weight and its exudate volume was reduced significantly due to the administration of standard drug indomethacin. But administration of earthworm extract was found to exhibit better results in a dose dependent manner. Administration of 200 mg/ kg earthworm extract was found to reduce the above parameters and brought to near normalcy and this results was found to be followed by administration of 50 and 100 mg /kg, respectively.

Di Long “earth dragon” extract in traditional Chinese medicine (TCM) is made from red earthworms (*Lumbricus rubellus*). This TCM preparation is used empirically to treat high fever with convulsion and inflammatory joint pain. The ethanolic extract of L. rubellus powder contains notable amounts of phenolic acid and shows an antioxidant effect in vitro. L. rubellus powder can be potentially used as a natural antioxidant source to treat disorders associated with inflammation and oxidative stress.[29]

- ***Aduthinna paalai* (*Aristolochia bracteata*) [30]**

The ethanolic extract of the shade dried leaves of *A. bracteata* was evaluated for its anti inflammatory activity in wistar rats by using the carrageeenan induced left hind paw edema method. Significant reduction of edema volume was observed in the drug treated group when compared with the standard and untreated control. Antioxidant investigation of the ethanol extract along with its two successive fractions using nitric oxide and 1,1-diphenyl-2 picryl hydrazyl (DPPH)-induced free radical assay methods showed good free radical scavenging activity, thereby supporting its anti inflammatory properties.

Anti Pyretic Activity

- ***Poonagam* (Earthworm)[28]**

M.Balamurugan et al., studied the anti pyretic activity of earthworm extract by using the Brewer's yeast induced pyrexia in rats. The subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 24h of administration to rats. Treatments with earthworm extracts at the doses of 50, 100, and 200 mg/ kg decreased the rectal temperature in a dose dependent manner. The anti pyretic effect started from the first hour and was maintained for 4h, after administration of earthworm extract. The result obtained from both paracetamol and earthworm extract treated rats were compared with the control group and a significant reduction in the yeast induced elevated rectal temperature was observed.

- ***Aduthinna paalai* (*Aristolochia bracteata*)[31]**

Pet. ether and acetone extracts of *A. bracteata* was investigated for their anti pyretic activity . Pet. ether and acetone extracts of the plant *A. bracteata* were prepared using Soxhlet extraction. Pyrexia produced in rats by injecting 20ml/kg (s.c) of 20% aqueous suspension of Brewer's yeast suspension. Extracts at 250 mg/kg exhibited significant anti pyretic activity. Aspirin (300mg/kg) was used as standard for which Pet. Ether extracts was found to be more effective than acetone extract.

5. STANDARDIZATION OF THE DRUG[32]

5.1 STANDARDIZATION OF THE DRUG *POONAGA PARPAM* AS PER SIDDHA CLASSICAL LITERATURE[22]:

- **Analysis as per classical Siddha literature:**
    - Floating on Water
    - Fine enough to enter the crevices of finger
    - Irreversible reaction
    - Tasteless
    - Lusterless

1. Floating on Water:

A pinch of *Parpam* gently placed on the still surface of water in a vessel, did not sink immediately. It was found that the *Poonaga Parpam* particles floated over the surface of water indicated lightness of the trial.

2. Fine enough to enter the crevices of finger :

Parpam in well prepared form should be fine. When taken between thumb and index finger, the fine powder will fill up the lines of the finger print. A pinch of *Poonaga Parpam* was taken in between the thumb and index finger and rubbed. It was found that the *Poonaga Parpam* entered into the lines of the finger, and was not easily washed out from the lines, confirmed its fineness.

3. Irreversible reaction:

The well prepared *Parpam* does not reversible to its metallic state when heated with a mixture of cane jaggery, hemp powder, ghee and honey. A pinch of *Poonaga Parpam* was taken and mixed with cane jaggery, ghee and honey. It was observed that the *Poonaga Parpam* did not reversible to its metallic state.

4. Tasteless:

The well prepared *Parpam* should be completely tasteless .Presence of any taste like sweet or bitter indicate incomplete preparation which needed another Calcination process. When a small amount of *Poonaga Parpam* was kept on the tip of the tongue, no specific taste was found.

5. Lusterless:

If any shining particles present in *Parpam*, it indicates that the *Parpam* is not manufactured properly and contains unchanged substances like minerals, metals and other toxic substances. There should be no shining particles present in the well manufactured *Parpam*. The *Poonaga Parpam* was taken in a Petri bowl and observed for any luster in daylight through magnifying glass. No luster was observed in the *Poonaga Parpam*

The results were tabulated in **Table.No.1**

Finger print test

Floating on water test

Fig.No.6. Standardization of *Poonaga parpam* as per Siddha literature

5.2. STANDARDIZATION OF THE DRUG *POONAGA PARPAM* BY USING MODERN TECHNIQUES:

Standardization of drugs helps to prove its identity and determination of its quality and potency. Standardization of the siddha formulation is based on the qualitative and quantitative analysis through physico-chemical investigations and instrumental analysis.

As per AYUSH protocol for standardization, the following parameters were evaluated

- **Organoleptic characters**
  - Colour
  - Odour
  - Taste
  - State of matter
  - Consistency
  - Shape
  - Size
- **Physicochemical analysis**
  - Determination of Ash Values
  - Determination of Extractive Value
  - Physical characterization
- **Chemical analysis**
  - Preliminary Basic and Acidic radical studies
- **Instrumental Analysis**
  - Fourier Transform Infra-Red Spectroscopy (FTIR)
  - Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES)
  - Scanning Electron Microscope with Energy Dispersive X-Ray Analysis (SEM with EDAX)
  - X-Ray Diffractometer (XRD)
  - Thermo Gravimetric Analysis or Thermal Gravimetric Analysis (TGA)

The physico-chemical analysis of the test drug *"Poonaga Parpam"* have been done at The Tamil Nadu Dr.M.G.R. Medical University.

Identification of organic, polymeric and inorganic functional groups was engaged by using modern analytical technique Fourier Transform Infra-Red Spectroscopy (FTIR). The particle size (Detailed high resolution images), Identification and quantitative analysis of chemical elements of *Poonaga Parpam* were assessed by Scanning Electron Microscope with Energy Dispersive X-Ray Analysis (SEM with EDAX).

Detection of trace metals was carried out by using an analytical technique Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and Characterization and Identification of crystalline materials by X-Ray Diffractometer (XRD). The thermal analysis of the sample measured over time as the temperature changes by Thermo Gravimetric analysis or Thermal Gravimetric analysis (TGA). The above mentioned elemental analysis have been done at IIT Madras, SAIF. (FTIR, ICPOES, XRD, TGA) and Madurai Kamaraj University (SEM with EDAX)

5.2.1 ORGANOLEPTIC CHARACTER

Colour

The *Poonaga Parpam* was taken into watch glasses and placed against white back ground in white tube light. It was observed for its colour by naked eye.

Odour

The *Poonaga Parpam* was smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste

Small amount of *Poonaga Parpam* was kept over the tip of the tongue

The results were tabulated in **Table.No.2**

5.2.2 PHYSICO CHEMICAL ANALYSIS [33]

Physicochemical Properties of *Poonaga Parpam* was analyzed at The Tamil Nadu Dr. MGR Medical University, Anna Salai, Guindy, Chennai-600032.

Physico-chemical studies of the test drug is necessary for standardization, as it helps in under-standing the significance of physical and chemical properties of the substance being analyzed in terms of their observed activities and especially for the determination of their purity and quality. The analysis includes the determination of ash value, Loss on drying of the sample at 105ºC, pH value and Extractive value. These were carried out as per guidelines.

1. Loss on drying of the sample at 105ºC

4g of *Poonaga Parpam* was weighed in a previously weighed 100ml beaker and heated in an oven at 105ºC for 5hours. Cooled in a dessicator and weighed. Repeated the procedure till constant weight was obtained. The percentage loss in weight of the test drug was calculated by the following formula.

Calculation:

$$\text{Percentage of Loss on Drying at 105°C} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the test drug taken}} \times 100$$

2.Ash content

2.a. Total ash content

4g of *Poonaga Parpam* was weighed accurately in a previously ignited and tared silica dish. The material was evenly spread and ignited in a muffle furnace at 600°C until it became white indicating the absence of carbon. The dish was cooled in a dessicator and weighed. As carbon free ash cannot be obtained in this manner, the dish was cooled and the residue moistened with sufficient quantity of water. Dried on a water bath and then ignited in the electric furnace to get the constant weight. Cooled the dish in a dessicator and then weighed. The percentage of total ash of air-dried materials was calculated as per the formula given below.

Calculation:

$$\text{Percentage of total ash} = \frac{\text{Weight of the ash}}{\text{Weight of test drug taken}} \times 100$$

2.b. Acid-insoluble ash

The total ash of *Poonaga Parpam* was found out as described above. To the dish containing the total ash was added 45 ml of 1: 5 hydrochloric acid in three portions of 13 ml each time. Boiled gently for 5 minutes and filtered. Collected the insoluble matter on an ashless filter paper (Whatman No.41) and washed with distilled water until the residue was free from acid. Transfer the filter paper containing the insoluble mater to the original dish. Dried and ignited to the constant weight. Cooled the dish in a dessicator, and then weighed. Calculation was made by given formula.

Calculation:

$$\text{Percentage of acid-insoluble ash} = \frac{\text{Weight of the acid-insoluble residue}}{\text{Weight of test drug taken}} \times 100$$

2.c. Water Soluble Ash

The above obtained ash was boiled for 5minutes with 25mL water. The insoluble ash was collected using filter paper and washed with hot water and transferred to the silica crucible then ignites for 15 minutes at temperature not exceeding 450°C. The silica crucible and residue were weighed until constant weight was attained for determination of weight of insoluble ash. The weight of the water soluble ash was determined by subtracting the weight of insoluble ash from the weight of total ash.

3. Extractive value of the test drug

4 g of *Poonaga Parpam* was weighed accurately in a glass stoppered flask. Added 100 ml of distilled water and shaken occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipetted out 25 ml of the filtrate in a pre weighed 100 ml beaker and evaporated to dryness on a water bath .Kept in an air oven at 105ºC for 6 hours. Cooled in a desiccator and weighed. Repeated the experiment twice, and taken the average value. The percentage of water soluble extractive was calculated by the formula given below.

Calculation:

$$\text{Percentage of water soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

3. a. Water-soluble extractive of the test drug

3g of *Poonaga Parpam* in a glass stoppered flask and add 100 mL of distilled water, shake occasionally for 6 h and then allow standing for 18 h, then filter rapidly taking care not to lose any solvent and pipette out 25 mL of the filtrate in a pre weighed 100 mL beaker and evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 h, cool in a desiccator and weighed. Repeat the experiment twice and take the average value

$$\text{Percentage of water soluble extractive} = \frac{\text{Weight of the extract}}{\text{Weight of the sample taken}} \times \frac{100}{25} \times 100$$

3. b. Alcohol-soluble extractive of the sample

4 g of *Poonaga Parpam* was weighed accurately in a glass stoppered flask. Added 100 ml of distilled alcohol (approximately 95%) and shaken occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipetted out 25 ml of the filtrate in a pre weighed 100 ml beaker and evaporated to dryness on a water bath.

Kept in an air oven at 105ºC for 6 hours and cooled in a desiccator and weighed. Repeated the experiment twice, and taken the average value. The percentage of alcohol soluble extractive was calculated by the formula given below.

Calculation:

$$\text{Percentage of alcohol soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of the sample taken}} \times \frac{100}{25} \times 100$$

4. Determination of pH:

5 g of *Poonaga Parpam* was weighed accurately and placed in clear 100 ml beaker. Then 50 ml of distilled water was added to it and dissolved well. After 30 minutes it was then applied in to pH meter at standard buffer solution of 4.0, 7.0, and 9.2. Repeated the test four times and average was recorded.

5. Solubility test:

A. A little amount of the sample was taken in a clean, dry test tube and then shaken well with distilled water.

B. A little amount of the sample was taken in a clean, dry test tube and then shaken well with con. Hcl and Con. H_2SO_4. Sparingly soluble character of the sample indicates the presence of Silicate.

The results were tabulated in **Table.No.3**

5.2.3 CHEMICAL ANALYSIS OF *POONAGA PARPAM*[34]

The chemical analysis of *Poonaga Parpam* was carried out in Bio chemistry lab, National Institute of Siddha.

S.no	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Physical Appearance of extract**	Dark brown in colour	
2.	**Test for Silicate** A 500mg of the sample was shaken well with distilled water.	Sparingly soluble	**Presence of Silicate**
3.	**Action of Heat** A 500mg of the sample was taken in a dry test tube and heated gently at first and then strong.	No White fumes evolved.	Absence of Carbonate
4.	**Flame Test** A 500mg of the sample was made into a paste with Con. HCl in a watch glass and introduced into non-luminous part of the Bunsen	Bluish green flame	**Presence of Copper**
5.	**Ash Test** A filter paper was soaked into a mixture of extract and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.	No Appearance of yellow colour flame	Absence of Sodium

ESTIMATION OF ACID AND BASIC RADICALS

Preparation of Extract:

5gm of sample was taken in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 10 minutes. Then it was cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. This preparation was used for the qualitative analysis of acidic/basic radicals and biochemical constituents in it.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
	I. Test For Acid Radicals		
1.	**Test For Sulphate** 2ml of the above prepared solution was taken in a test tube to this added 2ml of 4% dil ammonium oxalate solution	Cloudy appearance present	**Presence of Sulphate**
2.	**Test For Chloride** 2ml of the above prepared solution was added with 2ml of dil-HCl until the effervescence ceases off.	No Cloudy appearance was formed	Absence of Chloride
3.	**Test For Phosphate** 2ml of the solution was treated with 2ml of dil.ammonium molybdate solution and 2ml of Con.HNo3	No Cloudy appearance was evolved.	Absence of Phosphate
4.	**Test For Carbonate** 2ml of the solution was treated with 2ml dil. magnesium sulphate solution.	Cloudy appearance was evolved.	**Presence of Carbonate**
5.	**Test For Nitrate** 1gm of the solution was heated with copper turning and concentrated H_2SO_4 and viewed the test tube vertically down.	No Brown gas was evolved	Absence of Nitrate
6.	**Test For Sulphide** 1gm of the solution was treated with 2ml of Con. HCL	No rotten egg smelling gas was evolved	Absence of Sulphide
7.	**Test For Fluoride & Oxalate** 2ml of solution was added with 2ml of dil. Acetic acid and 2ml dil. calcium chloride solution and heated**.**	No cloudy appearance.	Absence of Fluoride and Oxalate

8.	**Test For Nitrite** 3drops of the solution was placed on a filter paper, on that-2 drops of dil.acetic acid and 2 drops of dil. Benzidine solution were placed.	No characteristic changes were noted.	Absence of Nitrite
9.	**Test For Borate** 2 Pinches (50mg) of the solution was made into paste by using dil.sulphuric acid and alcohol (95%) and introduced into the blue flame.	No Appearance of bluish green colour.	Absence of Borate
II. Test For Basic Radicals			
1.	**Test For Lead** 2ml of the solution was added with 2ml of dil. potassium iodine solution.	No Yellow precipitate was obtained	Absence of Lead
2.	**Test For Copper** One pinch (25mg) of solution was made into paste with Con. HCl in a watch glass and introduced into the non-luminuous part of the flame.	No blue colour appeared	Absence of Copper
3.	**Test For Aluminium** To the 2ml of solution dil. sodium hydroxide was added in 5 drops to excess.	No yellow Colour appeared	Absence of Aluminium.
4.	**Test For Iron** a. To the 2ml of solution, added 2ml of dil. ammonium solution b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNO_3 were added	Mild Red colour appeared	**Presence of Iron**
5.	**Test For Zinc** To 2ml of the solution dil. sodium hydroxide solution was added in 5	No White precipitate was formed	Absence of Zinc

	drops to excess and dil. A mmonium chloride was added.		
6.	**Test For Calcium** 2ml of the solution was added with 2ml of 4% dil.ammonium oxalate solution	Cloudy appearance and white precipitate was formed	**Presence of calcium**
7.	**Test For Magnesium** To 2ml of solution dil. sodium hydroxide solution was added in 5 drops to excess.	No White precipitate was obtained	Absence of Magnesium
8.	**Test For Ammonium** To 2ml of solution 1 ml of Nessler's reagent and excess of dil.sodium hydroxide solution were added.	No Brown colour appeared	Absence of Ammonium
9.	**Test For Potassium** A pinch (25mg) of solution was treated with 2ml of dil. sodium nitrite solution and then treated with 2ml of dil. cobalt nitrate in	No Yellow precipitate was obtained	Absence of Potassium
10.	**Test For Sodium** 2 pinches (50mg) of the solution was made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow colour flame evolved.	Absence of Sodium
11.	**Test For Mercury** 2ml of the solution was treated with 2ml of dil. sodium hydroxide	No Yellow precipitate was obtained	Absence of Mercury
12.	**Test For Arsenic** 2ml of the solution was treated with 2ml of dil. sodium hydroxide solution.	No Brownish red precipitate was obtained	Absence of Arsenic

III. Miscellaneous			
1.	**Test For Starch** 2ml of solution was treated with weak dil.Iodine solution	No Blue colour developed	Absence of starch
2.	**Test For Reducing Sugar** 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the solution and again boil it for 2 minutes. The colour changes were noted.	No Brick red colour is developed	Absence of reducing sugar
3.	**Test For Alkaloids** a) 2ml of the solution was treated with 2ml of dil.potassium lodide solution. b) 2ml of the solution was treated with 2ml of dil.picric acid. c) 2ml of the solution was treated with 2ml of dil.phosphotungstic acid.	No Yellow colour developed	Absence of of Alkaloid
4	**Test For Tannic Acid** 2ml of solution was treated with 2ml of dil. ferric chloride solution	No Blue-black precipitate was obtained	Absence of Tannic acid
5	**Test For Unsaturated Compound** To the 2ml of solution , 2ml of dil. Potassium permanganate solution was added.	Potassium permanganate was not decolourised	Absence of unsaturated compound
6	**Test For Amino Acid** 2 drops of the solution was placed on a filter paper and dried well. 20ml of Burette reagent was added**.**	No Violet colour appeared	Absence of amino acid

7	**Test For Type of Compound** 2ml of the solution was treated with 2 ml of dil. ferric chloride solution.	No green and red colour developed No Red colour developed No Violet colour developed No Blue colour developed.	Absence of quinole pinephrine and pyrocatecho l Absence of Antipyrine, Aliphatic amino acid and meconic acid. Apomorphine salicylate and Resorcinol were absent Morphine, Phenol cresol and hydrouinone were Absent.

Results were discussed in **Table.No.4**

5.2.4. FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)[35,36]

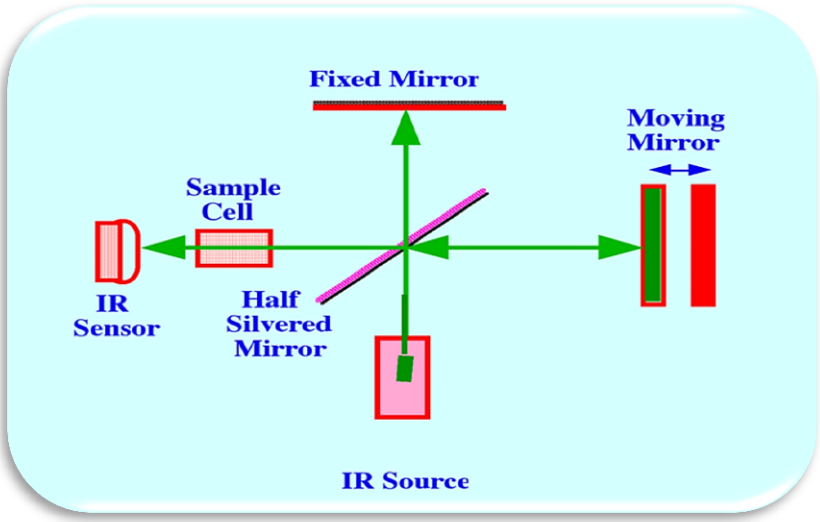
Instrument details:

Model	: Perkin Elmer- Spectrum one: FT-IR Spectroscopy
Scan Range	: MIR 450-4000 cm-1
Resolution	: 4cm-1

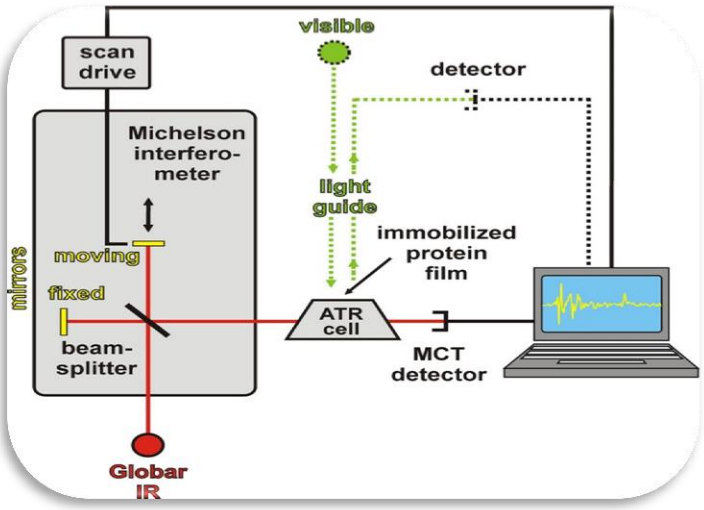
Principle:

Fourier Transform Infrared Spectroscopy (FTIR) is an analytical technique used to identify mainly organic materials. FTIR analysis results in absorption spectra which provide information about the chemical bonds and molecular structure of a material. The FTIR spectrum is equivalent to the "fingerprint" of the material and can be compared with cataloged FTIR spectra to identify the material.

Fourier Transform Infrared Spectroscopy (FTIR)



FTIR MECHANISM



Mechanism of FTIR analyzer

Fourier Transforms Infrared Spectroscopy analytical capabilities:

- Identifies chemical bond functional groups by the absorption of infrared radiation which excites vibrational modes in the bond
- Especially capable of identifying the chemical bonds of organic materials
- Detects and Identifies organic contaminants
- Identifies water, phosphates, sulphates, nitrates, nitrites, and ammonium ions

- Detection limits vary greatly, but are sometimes $<10^{13}$ bonds/cm^3 or sometimes sub monolayer
- Useful with solids, liquids, or gases.

Applications:

- Infrared spectrum is useful in identifying the functional groups like -OH, -CN, -CO, -CH, -NH2, etc. Also quantitative estimation is possible in certain cases for chemicals, pharmaceuticals, petroleum products, etc. Resins from industries, water and rubber samples can be analyzed.

Sample preparation method:

FT-IR spectra were recorded at SAIF, IIT Madras, India. The Perkin Elmer Spectrum One Fourier Transform Infrared (FTIR) Spectrometer was used to derive the FT IR Spectra of *Poonaga Parpam* in Potassium Bromide (KBr) matrix with scan rate of 5 scan per minute at the resolution 4cm-1 in the wave number region 450-4000cm-1. The samples were grounded to fine powder using agate motor and pestle and then mixed with KBr. They were then Pelletized by applying pressure to prepare the specimen (the size of specimen about 13 mm diameter and 0.3 mm in thickness) to recorded the FT- IR Spectra under Standard conditions. FT- IR Spectra were used to determine the presence of the functional groups and bands in the *Poonaga Parpam*. The recorded spectrum shown in Fig.No.7

5.2.5. ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)[37]

Instrument details:

Manufacturer: Perkin Elmer

Model: Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer (ICP)

Principle:

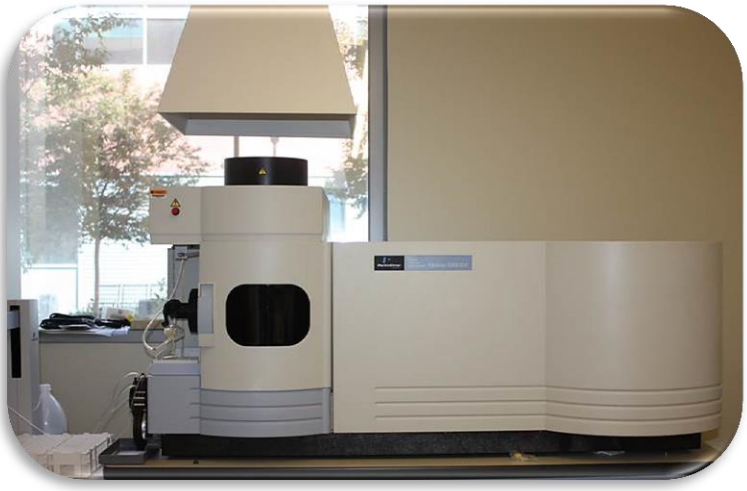
In this technique, the high temperature plasma source atomizes the sample and excites the atoms resulting in emission of photons. The atoms of each element in the sample emit specific wavelength of light. The emission spectrum from the plasma is dispersed by an optical spectrometer, so that intension of the individual wavelength can be measured. The number of photos emitted is directly proportional to the concentration of the element. The photos may be detected either sequentially or simultaneously. Quantitative analysis is achieved by measuring the intensity of these specific wavelength and after performing the calibration using known standards. Identifying the presence of emission at the wavelength characteristic of the element of interest obtaining quantitative information i.e how much of an element is in sample can be accomplished using plots of emission intensity versus concentration called calibration curves.

Application:

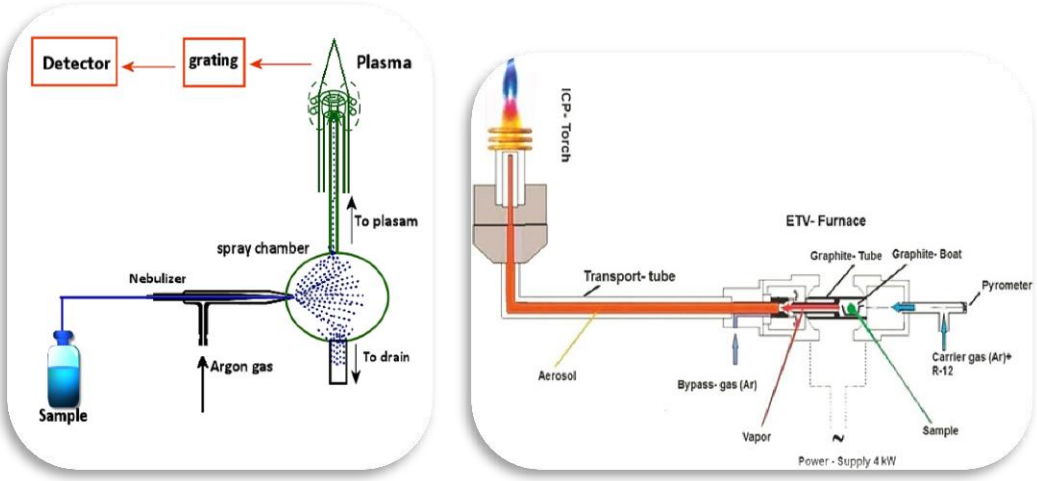
The analysis of major and minor elements in solution samples.

Objectives:

- Determine elemental concentrations of different metals.
- Learn principles and operation of the ICP-OES instrument
- Develop and put on a method for the ICP-OES sample analysis
- Enhance the instrumental conditions for the analysis of different elements
- Probes the outer electronic structure of atoms



ICP-OES ANALYSER (Perkin Elmer Optima 5300 DV)



Mechanism of ICP-OES analyzer

Mechanism:

In plasma emission spectroscopy (OES), a sample solution is presented into the core of inductively coupled argon plasma (ICP), which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. This light is collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light is then collected by wavelength and amplified to yield an strength of

measurement that can be converted to an elemental concentration by comparison with standardization values.

Sample preparation – Microwave Digestion

The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was done in SAIF, IIT MADRAS, Chennai-36 using Perkin Elmer Optima 5300 DV.

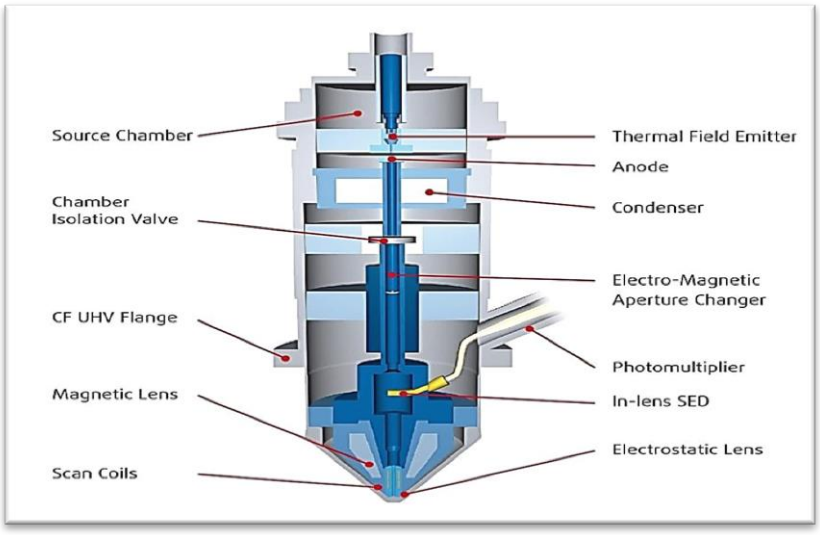
- Weigh 0.37 g of test sample and transfer into a liner provided with instrument.
- Slowly add 9ml of Nitric acid or Sulphuric acid such that no piece of sample sticks on the slide.
- Mix thoroughly and allow reacting for few minutes.
- Place the liner in the vessel jacket.
- Close the screw cap hand- tight in clockwise direction.
- Seal the vessel and placed in the rotor fixed in microwave.
- Set temperature to 180°C for 5 minutes, hold at 180°C for least 10 minutes.
- Allow the vessels to cool down to a vessel interior temperature below 60°C and to a vessel surface temperature (IR) below 50°C before removing the rotor.
- The digested sample was made up to 100ml with Millipore water.
- If visible insoluble particles exist, solution could be filtered through whatmann filter paper.
- Transfer the digested solution into plastic containers and label them properly.

5.2.6. SCANNING ELECTRON MICROSCOPY (SEM) WITH ENERGY DISPERSIVE X-RAY ANALYSIS (EDX)[38] :

Instrument details:

Model: The VEGA TESCAN 3 instrument is used with tungsten filament to image the samples.

SEM INSTRUMENT



SEM MECHANISM

Principle :

Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX) is the best known and most widely-used of the surface analytical techniques. A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the sample's surface topography and composition. The electron beam is generally scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image. SEM can achieve resolution better than 1 nanometer. Specimens can be observed in high vacuum, in low vacuum, in wet conditions (in environmental SEM), and at a wide range of cryogenic or elevated temperatures.

The most common SEM mode is detection of secondary electrons emitted by atoms excited by the electron beam. The number of secondary electrons depends on the angle at which beam meets surface of specimen, i.e. on specimen topography. By scanning the sample and collecting the secondary electrons with a special detector, an image displaying the topography of the surface.

An **Energy Dispersive X-Ray Analyzer (EDX or EDA)** is widely used for elemental identification and quantitative compositional information of the test substances. Scanning electron microscope provides high quality images with ~X50, 000 magnifications allowing up to nano particles size to be seen.

Applications:

- Identification of metals and materials
- Particle contamination identification and elimination
- Classification of materials
- Examination of surface morphology (including stereo imaging)
- Analysis and identification of surface and airborne contamination
- Powder morphology, particle size and analysis

Sample preparation method:

All samples must be of an appropriate size to fit in the specimen chamber and are generally mounted rigidly on a specimen holder called a specimen stub. For conventional imaging in the SEM, specimens must be electrically conductive, at least at the surface, and electrically grounded to prevent the accumulation of electrostatic charge at the surface. Nonconductive specimens tend to charge when scanned by the electron beam, and especially in secondary electron imaging mode, this causes scanning faults and other image artifacts. They are therefore usually coated with an ultrathin coating of electrically conducting material, deposited on the sample either by low-vacuum sputter coating or by high-vacuum evaporation.

Coating:

- For best images, and to avoid charging of instances, it is desirable to gold or carbon coat the sample prior to examination.
- Au coating indeed generates Au particles, whose sizes increase significantly with coating time. Two minutes coating may have particles in the range of 5~20 nm.

In the present study, A representative portion of each sample was sprinkled onto a double side carbon tape and mounted on aluminum stubs in order to get a high quality secondary electron image for SEM examination.

The sample is coated with gold in vacuum chamber by adopting suitable reaction conditions. The gold coated is further used to record SEM images. The SEM instrument is also connected with EDX tool for analyzing the presence of atomic elements in the sample.

SEM picture and EDX results of *Poonaga Parpam* was shown in **Fig.No.7 and 8**

EDX results of *Poonaga Parpam* was represented in **Table.No.7**

5.2.7. X-RAY DIFFRACTOMETRY (XRD) [39]:

Instrument details

Model : Bruker discover D8 X ray diffractometer.

X- Ray Diffraction:

The x-ray diffraction technique is used as a basic characterization tool for different materials. This technique has appeared as powerful tool for determining the crystal structure chemical analysis, stress measurements, phase equilibria and particle size. The XRD pattern is the fingerprint of a crystalline material as this technique gives information on the phase, purity, and structure of a material.

X-RAY DIFFRACTOMETRY (XRD)

Principle:

X-ray powder diffraction is most widely used non-destructive technique for investigation of structural properties of crystalline materials. X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample.

The spacing of atoms in crystal lattices is of the same order as the wavelength of X-radiation (0.1 to 100 Angstrom). Von Laue discovered (1912) that a crystal could be used as a diffraction grating for X-rays.

William Lawrence Bragg discovered (1912) law relating the spacing between atoms in a crystal to the angle at which X-rays are scattered when they strike the crystal. Bragg equation relates the spacing between adjacent crystal planes and the θ angle of diffraction.

$$n\lambda = 2d \sin \theta$$

Where, n = integer determined by the order given, d = spacing between crystal planes ,θ = angle of scattering, λ=wavelength of x-ray beam , θ= diffraction angle

Applications:

Quantitative analysis of organic minerals, metals and alloys, crystallographic studies.

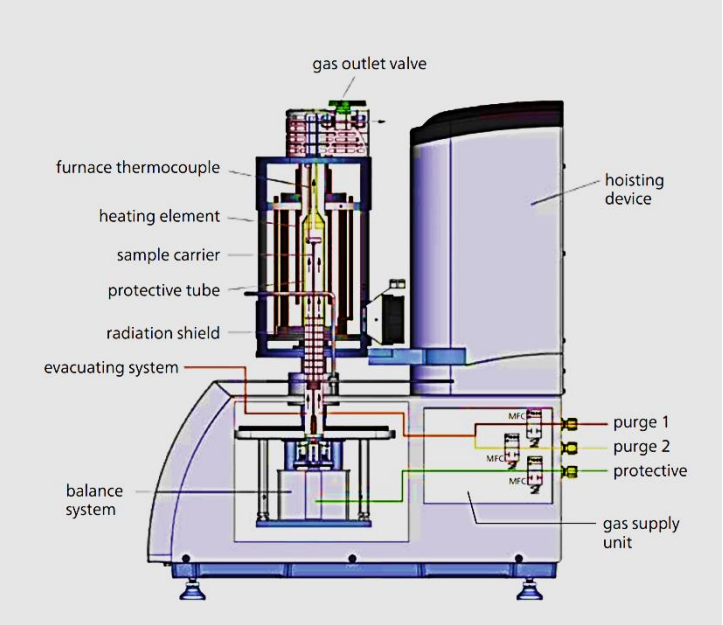
Sample Preparation method:

Poonaga parpam were characterized using a X-ray diffractormeter (D8 Focus, Bruker) equipped with a photo scintillation detector at IIT Madras, Saif. Angular range (2θ=10-60°), rate 0.01°/sec. 200mg of sample was taken. Sample was grinded to fine powder. Powder less than ~10 μm (or 200-mesh) in size is preferred. The powder was mounted on sample holder. Gently pressed the powder flush with the sample holder using a glass slide. Removed excess powder from the sample holder edges and carefully placed in the appropriate XRD slot and subjected for reading.

5.2.8.THERMOGRAVIOMETRIC ANALYSIS (TGA) [40]:

Instrument details

Model: STA 449 F3 Jupiter



Thermo Gravimetric Analysis (TGA)

Principle:

Thermo Gravimetric Analysis (TGA) is an analytical technique used to determine a material's thermal stability and its fraction of volatile components by monitoring the weight change that occurs as a specimen is heated. The measurement is normally carried out in air or in an inert atmosphere, such as helium or argon, and the weight is recorded as a fraction of increasing temperature. Sometimes, the measurement is performed in a lean oxygen atmosphere (1 to 5% O_2 in N_2 or He) to slow down oxidation.

In addition to weight changes, some instruments also record the temperature difference between the specimen and one or more reference pans (differential thermal analysis, or DTA) or the heat flow into the specimen pan compared to that of the reference pan (Differential Scanning calorimetry or DSC). The latter can be used to monitor the energy released or absorbed via chemical reactions during the heating process. In the particular case of carbon nanotubes, the weight change in an air atmosphere is typically a superposition of the weight loss due to oxidation of carbon

into gaseous carbon dioxide and the weight gain due to oxidation of residual metal catalyst into solid oxides.

Applications:

Current areas of applications of thermal analysis include environmental measurements, compositional analysis, product reliability, stability, chemical reactions, and dynamic properties. TGA has been used in the kinetic analysis of polymer stability, compositional analyses of multicomponent materials, atmospheric analyses and corrosion studies.

Thermal analysis of *parpam*:

- To differentiate between different polymorphic structures
- To investigate the transformations which occur during the polymorphic transformation by using different heating rates
- To determine polymorphic purity
- Compositional analysis
- To investigate thermal stability and the effect of addictives
- To measure the unreacted organic species content of herbo- metallic preparations by heating a sample and to remove the resin by application of heat
- A better understanding of the influence of temperature on the properties of herbo- metallic preparations
- To optimize processing conditions and improve product quality.

Sample preparation method:

Thermo Gravimetric analysis was carried out in IIT madras, SAIF. Good thermal contact between the sample and heat- flux sensor is an indispensable requirement for optimum results. 12.4 mg of the sample is evenly distributed in the bottom of the sample crucible. When filling the crucible, no sample material may remain on the edge of the crucible. The sample crucible was placed on the front- hand sample support and subjected for reading.

6.1. EVALUATION OF BRONCHODILATOR ACTIVITY OF *POONAGA PARPAM* USING HISTAMINE INDUCED BRONCHO CONSTRICTION IN GUINEA PIG[41,42]

Aim:

To study the Broncho dilator activity of *Poonaga parpam* by histamine induced bronchoconstriction in Guinea pig .

Materials and methods:

Test Substance	:	*Poonaga Parpam*
Animal Source	:	TANUVAS, Madhavaram, Chennai.
Animal	:	Albino Guinea pig
Body Weight	**:**	700 gms
Acclimatization	**:**	14 days prior to dosing.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Diet	**:**	Pellet feed
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animal was housed in Polypropylene cage provided with bedding of husk.
Housing temperature	**:**	25±2°C
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.

Selection of animals:

Healthy albino guinea pig weighing 700 gms of male sex was used in this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory IAEC approved no: IAEC/ LI/23/CLBMCP/2017.

The animal was kept in plastic cage and maintained under controlled environment (temperature 25±2°C and 12hrs dark and light cycle) with standard diet, water *ad libitum* during experiment. The animal was allowed an acclimatization period of 14 days before actual experiment. The animal experiment was performed with accordance to legislation on welfare.

PROCEDURE[43]

Histamine was dissolved in distilled water to prepare 0.2% w/v solution. Overnight fasted Guinea pigs with free access to water were divided into four groups each containing 6 animals.

- Group-I was treated as Vehicle Control- Honey (p.o)
- Group-II received standard drug Chlorpheniramine maleate (2 mg/kg , i.p)
- Group-III *Poonaga parpam* (100mg/kg, p.o)
- Group-IV *Poonaga parpam* (200mg/kg, p.o)

All the doses were given orally once a day for 5 days. Prior to drug treatment each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol using a ultra-sound nebulizer in an aerosol chamber under constant pressure of 40mm/Hg. The required time for appearance of pre convulsive dyspnoea produced by the histamine was noted for each animal. The pre convulsive time (PCT) was determined from the time of exposure to onset of dyspnoea leading to the appearance of convulsions. As soon as the PCT were noted, the animal were removed from the chamber and placed in fresh air for recovery. This time for pre convulsive dyspnoea was recorded as basal value. *Guinea pigs* were then allowed to recover from dyspnoea for 2 days. Animals in group 1 served as vehicle control and received honey. The animals of group 2 received the standard drug - Chlorpheniramine maleate intraperitoneally and group 3 & 4 were given, by oral intubation, 100 and 200mg/kg of the test drug *Poonaga parpam*, respectively. In order to observe the Broncho dilator effect of the test substance on induced broncho contractions, the test material *Poonaga parpam* was added in a cumulative fashion (100 mg and 200 mg) to obtain the concentration-dependent inhibitory responses. These animals were again subjected to histamine aerosol after 1hr of drug administration and PCT was determined. The protection offered by treatment was calculated by using the formula

Percentage Protection = (1 - T1/T2) X 100 Where,

T1 = the mean of PCT before administration of test drugs.

T2 = the mean of PCT after administration of test drugs [44].

6.2. EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *POONAGA PARPAM* IN WISTAR ALBINO RATS[45]

Aim:

To study the Anti-inflammatory effect of *Poonaga Parpam* in Wistar albino rats by Carrageenan-induced rat paw edema.

Materials and methods:

Test Substance	**:**	***Poonaga Parpam***
Animal Source	**:**	TANUVAS, Madhavaram, Chennai.
Animals	**:**	Wistar Albino Rats (Male -12, Female -12)
Age	**:**	6-8 weeks
Body Weight	**:**	140-160gm.
Acclimatization	**:**	14 days prior to dosing.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	By cage number, animal number and individual marking by using Picric acid.
Diet	**:**	Pellet feed
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	24-28°C
Relative humidity	**:**	between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.

Selection of animals:

Healthy Wistar albino rats (140- 160g) of both sexes were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory. IAEC approved no: NIS/IAEC-III/03/29092016.

The animals kept in plastic cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water *ad libitum*. They were fed with standard diet and kept in well ventilated animal house and they were also maintained with alternative dark-light cycle of 12hrs throughout the studies. Rats were allowed an acclimatization period of 14 days before actual experiments. The rats were closely observed for any infection and if they show any signs of infection they were excluded from the study. The animal experiment was performed with accordance to legislation on welfare.

The experimental protocol[46,47]

Animal grouping:

Both sex of Adult wistar Albino rats weighing (140-160g) were used in this study. Rats were divided into 4 groups, consisting six animals for each group.

Group I : Vehicle control - received only honey orally

Group II : Received Standard drug Indomethacin (10mg/kg orally)

Group III : Received *Poonaga Parpam* (12 mg/kg orally)

Group IV : Received *Poonaga Parpam* (24 mg/kg orally)

Acute anti inflammatory effect was evaluated by carrageenan-induced hind paw edema. Carrageenan was administrated by sub-plantar injection of 0.1 ml freshly prepared 1% suspension in right hind paw in rats. Group II, III, and IV of animals were pretreated with 10 mg/kg body weight standard drug Indomethacin, *Poonaga Parpam* 12 mg/kg and 24 mg/kg at 1hr before eliciting paw edema. Rat's paw volume was measured initially and then 1,2,3 hrs after the carrageenan injection by using plethysmographic method.

The edema inhibitory activity was calculated according to the following formula-

Edema (%) inhibition = (1-D/C) 100

Where,

D-represents the percentage difference in increased paw volume after the administration of test drugs to the rats.

C-represents the percentage difference of increased volume in the control groups.

Statistical analysis

All the results were reported as mean ± SD. They were further analyzed using one way analysis of variables (ANOVA) followed by Dunnet's multiple comparison test.

6.3 EVALUATION OF ANTI-PYRETIC ACTIVITY OF *POONAGA PARPAM* IN WISTAR ALBINO RATS[48,49]

Aim

To study the Anti-Pyretic Activity effect of *Poonaga Parpam* in Wistar albino rats by Brewer's yeast induced pyrexia.

Materials and methods:

Test Substance	**:**	***Poonaga Parpam***
Animal Source	**:**	TANUVAS, Madhavaram, Chennai.
Animals	**:**	Wistar Albino Rats (Male -12, Female -12)
Age	**:**	6-8 weeks
Body Weight	**:**	140-160gm.
Acclimatization	**:**	14 days prior to dosing.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	By cage number, animal number and individual marking by using Picric acid.
Diet	**:**	Pellet feed
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	24-28°C
Relative humidity	**:**	between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.

Selection of animals:

Healthy Wistar albino rats (140- 160g) of both sexes were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory. IAEC approved no : NIS/IAEC-III/03/29092016.

The animals kept in plastic cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water *ad libitum*. They were fed with standard diet and kept in well ventilated animal house and they were also maintained with alternative dark-light cycle of 12hrs throughout the studies. Rats were allowed an acclimatization period of 14 days before actual experiments. The rats were closely observed for any infection and if they show any signs of infection they were excluded from the study. The animal experiment was performed with accordance to legislation on welfare.

The experimental protocol[50]

Animal grouping:

Both sex of Adult wistar Albino rats weighing (140-160g) were used in this study. Rats were divided into 4 groups, consisting six animals for each group.

Group I :Control -Honey plus yeast injection

Group II :Received Standard drug Paracetamol (150 mg/kg orally plus yeast injection)

Group III :Received *Poonaga Parpam* (12 mg/kg orally plus yeast injection)

Group IV :Received *Poonaga Parpam* (24 mg/kg orally plus yeast injection)

Brewer's yeast induced hyperpyrexia method

The animals were fasted overnight with free access to water prior to the experimental procedure. The normal temperature of each rat in four groups was measured rectally at one hour interval on a clinical thermometer.

Before yeast injection the basal rectal temperature of rats was recorded by inserting the clinical thermometer to a depth of 2 cm into the rectum and after recording animals were given subcutaneous injection of 10ml/kg of 20% w/v yeast

suspended in normal saline below the nape of the neck for elevation of body temperature of rats.

After 8 hours of yeast injection, rats which shows a rise in temperature of atleast 1° c were taken for the study. The honey was administered orally to the control groups of animals and paracetamol at the dose of 150mg/ml was administered orally to the standard groups of animals.

Poonaga Parpam was administered orally at a dose of 12 mg/kg and 24 mg/kg of body weight to Group –III and Group –IV respectively. Rectal temperature was recorded by clinical thermometer at 0, 1, 2, 3hrs after drug administration and tabulated.[51]

Evaluation of parameters

Anti pyretic activity was evaluated by comparing initial rectal temperature (°C) before yeast injection, with rectal temperature (°C) after 8 hours of yeast injection at different time intervals.

Statistical analysis

All the results were reported as mean ± SD. They were further analyzed using one way analysis of variables (ANOVA) followed by Dunnet's multiple comparison test.

7. RESULTS

Many studies have been carried out to bring the efficacy and potency of the drug ***Poonaga parpam***. The study includes literary collections, organoleptic character, physicochemical analysis, pharmacological and analytical studies. The drug *Poonaga parpam* has been selected from the text **"*Sikicha Rathina Deepam*".**

- Botanical aspect explains the active principle and medicinal uses of the plants.
- *Gunapadam* review brings the effectiveness of the drug in the management of Bronchial asthma.
- The pharmacological review explains about the evaluation of Broncho dilator, Anti inflammatory, Anti pyretic activities.

5. STANDARDIZATION OF THE DRUG

5.1 STANDARDIZATION OF THE DRUG *POONAGA PARPAM* AS PER SIDDHA CLASSICAL LITERATURE:

Siddhars used these following standardization methods to ensure the safety and efficacy of the *parpam*. It shows the effectiveness of the drug.

Table.No.1 Results of Siddha Standardization

S.No.	Parameter	Results of *Poonaga parpam*	Interpretation
1.	Floating on Water	Floats on water	Lightness of drug.
2.	Finger Print Test	Impinged in the furrow of fingers	Indicates fine particles of powder.
3.	Luster	Lusterless	Change of specific character of raw material after incineration
4.	Taste	No specific taste, Mild irritation is felt	Change of specific character of raw material after incineration

Interpretation:

1. **Floating on water:**

   The test drug which floats on water has less specific gravity. Thus *Poonaga parpam* possesses specific gravity less than the water.

2. **Finger print test**:

   Only the particles which are in micro fine size can enter into the furrows of the finger print. Finger print test indicates the presence of micro fine particles in *Poonaga parpam.*

3. **Lusterless & taste:**

   Poonaga parpam is lusterless and tasteless because there is no free metal present.

5.2 STANDARDIZATION OF THE DRUG *POONAGA PARPAM* BY USING MODERN TECHNIQUES:

Traditional remedies is advantageous, it does suffer some limitations. The main limitation is the lack of standardization of raw materials, of processing methods and of the final products, dosage formulation, and the non- existence of criteria for quality control. Standardization of the drug is more essential to derive the efficacy, potency of the drug by analyzing it through various studies. Following tables and charts are the results of physicochemical and chemical analysis. Physical characterization and estimation of basic and acidic radicals have been done and tabulated. Pharmacological activity and analytical studies of the drug were derived. Its results has been tabulated below.

5.2.1 ORGANOLEPTIC CHARACTERS:

Table.No.2. Organoleptic characters of *Poonaga parpam*

S.No	Parameter	Results
1	Colour	Dark brown coloured
2	Odour	Odourless
3	Taste	Tasteless
4	State of matter	Solid
5	Consistency	Powder

Table.No.3. Physicochemical characterization of *Poonaga parpam*

S.No	Parameters	Percentage
1	Loss on drying	Less than 1%
2.a	Total ash value	24.72%
2.b	Acid insoluble ash	0.72%
2.c	Water soluble ash	6.94%
3.a	Water soluble extraction	1.88%
3.b	Alcohol soluble extraction	3.54%
4	pH	8.4
5	Solubility	Soluble in acids (Hcl and H_2So_4)

Interpretation

The stability of a drug and its shelf–life are reliant on moisture content. Determination of moisture (Loss on drying) in a drug is one of the important tests in pharmaceutical analysis. The analytical parameters like total Ash value, Acid

insoluble ash value, Loss on drying values are helping us to interpret the digestion and solubility capacity of the drug.

Physico-chemical analysis of *Poonaga parpam* showed that Loss on drying (LOD) is less than 1% which shows that low moisture content present in the prepared medicine. Increased moisture content is the issue for instability of a drug and lesser shelf life of a drug. Since *Poonaga parpam* was well prepared, it could get maximum stability and better shelf life. Longer shelf life i.e., 100 years for *Parpam* mentioned in Siddha literature is justified from the above observation.

By the above results, the trial drug shows that total ash value was found to be 24.72% whereas the acid insoluble ash and water soluble ash was 0.72% and 6.94% respectively. The value of total ash in the formulation is high because of the presence of inorganic ingredients and the method of preparation of this drug is calcination procedure. Total ash value used to estimate the inorganic material such as silicate, carbonates, oxalates and phosphates.

The water soluble extractive values indicates the presence of sugar, acids. The alcohol soluble extractive values indicates the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids. Water soluble extractive and alcohol soluble extractive values of this formulation was 1.88% and 3.54% respectively.

pH of the trial drug was 8.4. It shows the alkalinity of the drug. The adjuvant of the drug is honey, which possesses acidic pH (3.4- 6.1). Hence, this adjuvant reduces the alkalinity of *Poonaga parpam*. According to pharmacokinetics, alkaline drugs are absorbed in alkaline environment i.e., the intestine.

The test drug is not soluble in water medium and well soluble in Hcl and H_2So_4.

5.2.3 CHEMICAL ANALYSIS OF *POONAGA PARPAM*

S.NO	Parameter	Result
1	Test for silicate	**Present**
2	Action on heat	-
3	Flame test	Bluish green flame indicates the presence of copper
4	Ash test	-

Table.No.4. Results of basic and acidic radical studies of *Poonaga parpam*

S.No	Parameter	Observation	Result
1	Test for Sulphate	Cloudy appearance present	Positive
2	Test for Chloride	-	Negative
3	Test For Phosphate	-	Negative
4	Test For Carbonate	Cloudy appearance was evolved.	Positive
5	Test For Nitrate	-	Negative
6	Test for Sulphide	-	Negative
7	Test For Fluride & oxalate	-	Negative
8	Test For Nitrite	-	Negative
9	Test For Borate	-	Negative

S.No	Parameter	Observation	Result
1	Test for Lead	-	Negative
2	Test for Copper	-	Negative
3	Test For Aluminium	-	Negative
4	Test For Iron	Mild Red colour appeared	Positive
5	Test For Zinc	-	Negative
6	Test for Calcium	Cloudy appearance and white precipitate was formed	Positive
7	Test For Magnesium	-	Negative
8	Test For Ammonium	-	Negative
9	Test For Potassium	-	Negative
10	Test For Sodium	-	Negative
11	Test For Mercury	-	Negative
12	Test For Arsenic	-	Negative

Miscellaneous

S.No	Parameter	Observation	Result
1	Test for starch	-	Negative
2	Test for reducing sugar	-	Negative
3	Test for alkaloids	-	Negative

4	Test for tannic acid	-	Negative
5	Test for unsaturated compound	-	Negative
6	Test for amino acid	-	Negative

The result of preliminary chemical analysis reveals that the trail drug *Poonaga parpam* has **Silicate, Copper, Sulphate, Carbonate, Iron, Calcium.**

5.2.4. FTIR

Fourier Transform Infra-Red Spectroscopy (FTIR) analysis results in absorption spectra provide information about the functional group and molecular structure of a material.

IR relates with the sample and the bonds among atoms in the molecule stretch and bend, absorbing infrared energy and creating the infrared spectrum. It is of two kinds bending and stretching. FT-IR is a very useful tool in the recognition of the functional groups of bio molecules, thus aiding in their structural elucidation, so confirming the presence of active molecules responsible for the therapeutic activity of Siddha drugs.

Fig.No.7 ***Poonaga parpam*** has following functional groups.

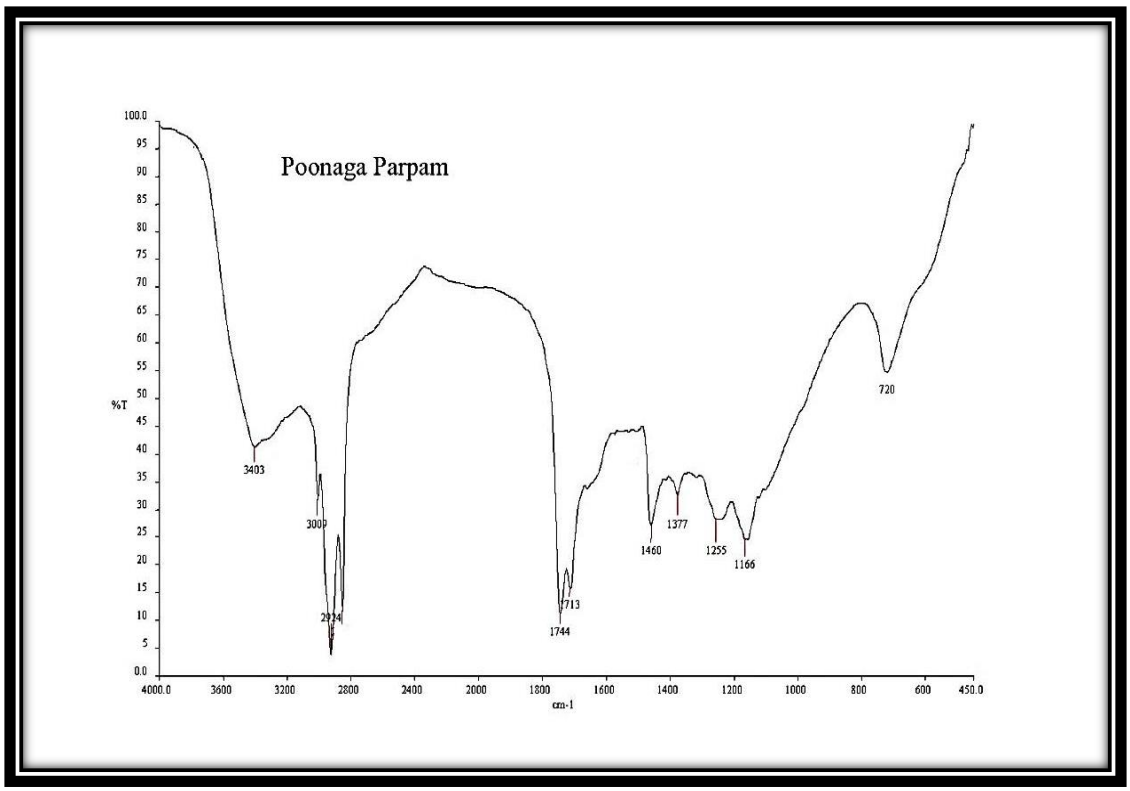


Table 5 INTERPRETATION OF FTIR SPECTRUM

Wave number (cm-1)	Vibrational modes of *Poonaga parpam* in IR region	Functional groups
3403	O-H Stretch	Alcohol
3009	C-H Stretch	Aromatic
2924	C-H Stretch	Alkane
1744	C=O Stretch	Carbonyl
1713	Acyclic Stretch	Ketone
1460	-C-H Bending	Alkane
1377	-C-H Bending	Alkane
1255	C-N Stretch	Amine
1166	C-F Stretch	Alkyl halide
720	C-Cl Stretch	Alkyl halide

- In the FT-IR Spectra analysis, this *Poonaga Parpam* sample exhibits the peak value shows in Table 5 at the wave number of 3403, 3009, 2924, 1744, 1713, 1460, 1377, 1255, 1166,720 having O-H stretch, C-H stretch, C=O stretch, acyclic stretch, -C-H bending, C-N stretch, C-F stretch, C-Cl stretch.
- This indicates the presence of some organic functional groups such as alcohol, aromatic, alkane, carbonyl, ketone, alkane, amine and alkyl halide.

- Stretching and bending modes of *Poonaga Parpam* shows the vibrational frequencies in the IR region. It confirms the dipole moment of the sample.
- From the observed FTIR Spectra the presence of some organic compounds are identified such as copper phosphate, potassium bicarbonate, copper selenite, ammonium thiosulfate, ammonium chloride, ammonium sulfate, ammonium chromate, sodium selenate and zinc chromate.

5.2.5. ICP-OES(INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

The drug *(Poonaga Parpam)* sample was analysed by the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to detect the trace elements and other elements quantitatively. The result of ICP-OES is given in the Table.No.6.

Table 6: ICP-OES of *Poonaga Parpam*

S.NO	Elements	Wavelength (nm)	*Poonaga parpam* (0.37010g)
1.	Al	396.152	BDL
2.	As	188.979	BDL
3.	Ca	315.807	12.080 mg/L
4.	Cd	228.802	BDL
5.	Cu	27.393	21.561 mg/L
6.	Fe	238.204	01.306 mg/L
7.	Hg	253.652	BDL
8.	K	766.491	03.021 mg/L
9.	Mg	285.213	01.204 mg/L
10.	Na	589.592	01.300 mg/L
11.	Ni	231.604	BDL
12.	Pb	220.353	BDL
13.	P	213.617	16.381 mg/L
14.	S	180.731	01.204 mg/L

BDL: Below Detectable Limit

1% = 10000ppm,

1ppm = 1/1000000 or 1ppm = 0.0001%

The toxic metals and the permissible limits

Heavy metals	WHO & FDA limits
Arsenic (As)	3 ppm
Mercury (Hg)	1ppm
Lead (Pb)	10ppm
Cadmium (Cd)	0.3ppm

Interpretation:

ICP-OES reveals high concentration of Cu in *Poonaga parpam* (21.561 mg/l). It also has physiologically important minerals like Ca, Fe, K, Mg, Na, P and S. From the above results, the heavy metals such as Arsenic, Cadmium, Lead, Mercury were observed as BDL and those are within the WHO permissible limits. Hence the safety of the drug *Poonaga parpam* is ensured for clinical use.

5.2.6. SEM (Scanning Electron Microscope with Energy Dispersive X-Ray Analysis):

In addition, the particle size and chemical elements were assessed by Scanning Electron Microscope with Energy Dispersive X-Ray Analysis (SEM EDAX). SEM is one of the most widely used instruments in research areas. The SEM picture of *Poonaga parpam* is shown in Fig.No.8

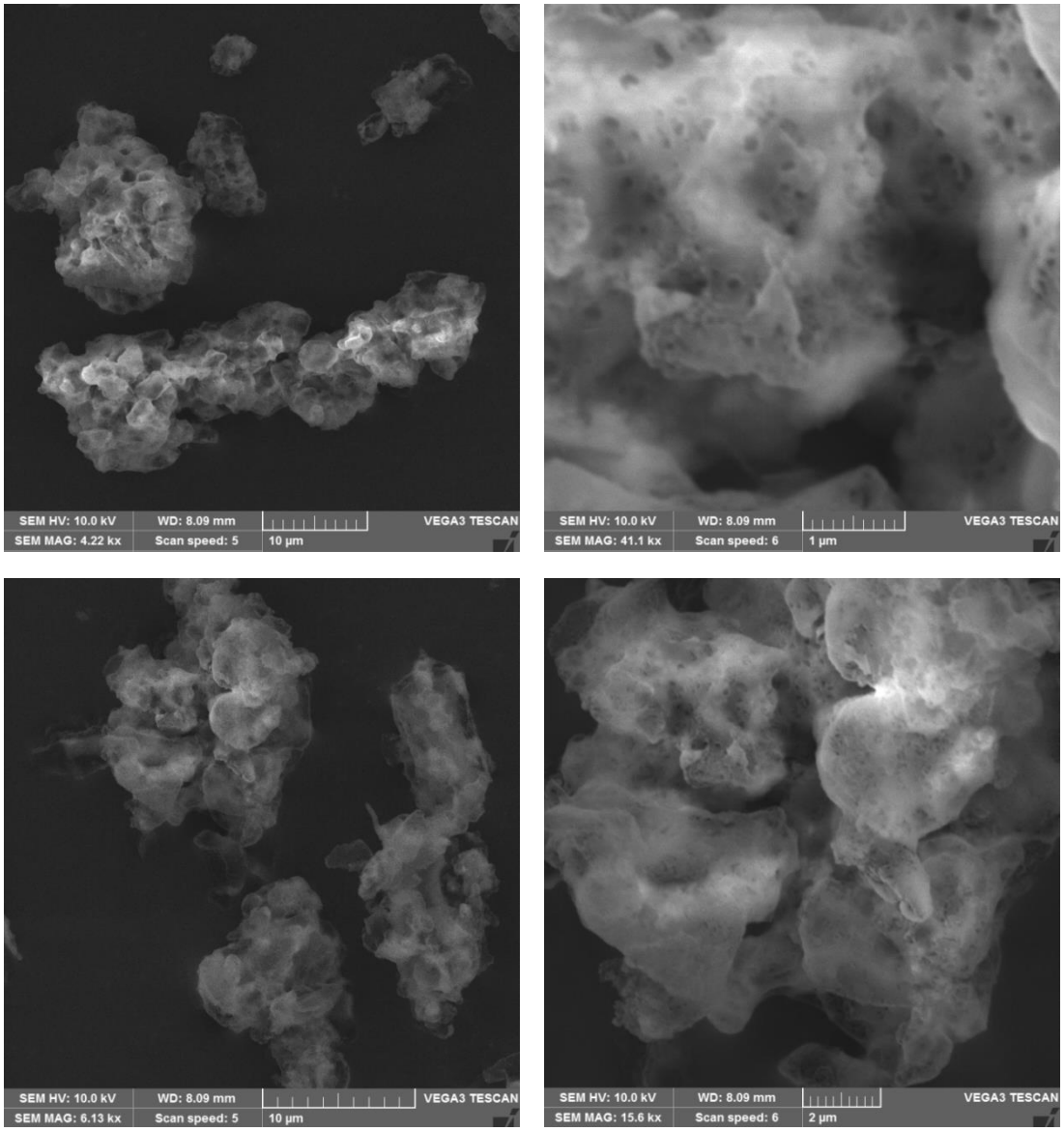


Fig.No.8. SEM image shows nano particles in *Poonaga Parpam*

EDAX

Energy Dispersive X-Ray Analysis is used to find out elements present in the sample qualitatively in a smaller area which is selected. The results of EDAX is shown in Fig.No.9 and Table.No.7

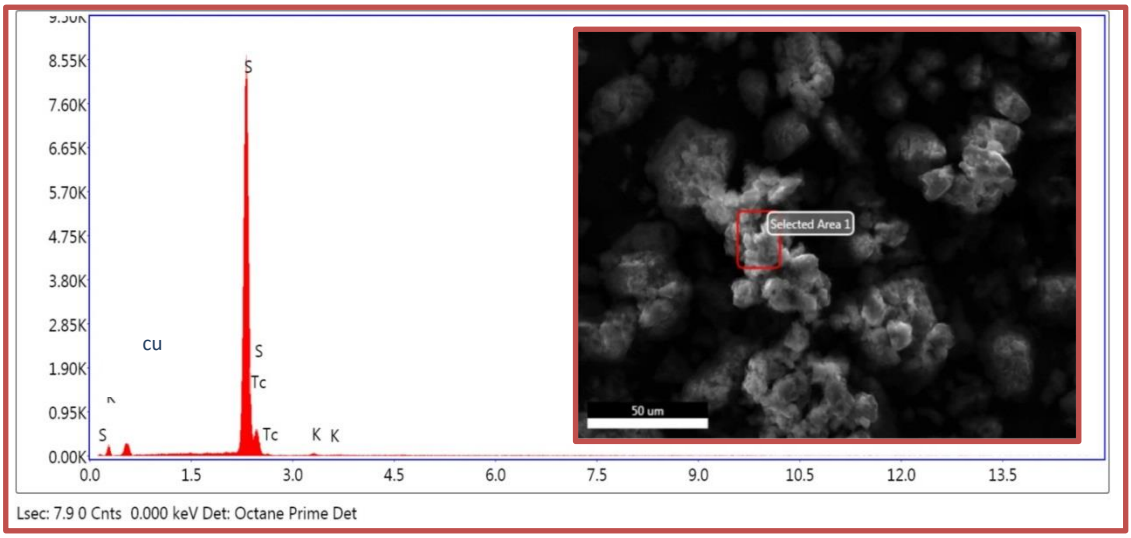


Fig.No.9 EDAX analysis of *Poonaga Parpam*

Table.No.7: Representing the weight and atomic percentage of elements present in *Poonaga Parpam*

Element	Weight %	Atomic %	Net Int.	Error %
Cu K	44.62	69.99	163.17	13.36
S K	48.65	28.59	9323.94	1.26
TcL	6.28	1.21	533.86	5.63
K K	0.45	0.22	48.41	21.64

Interpretation :

The SEM imaging of the *Poonaga Parpam* shows that the particles are in nano size and have pores in it as shown in Fig 8. They are nano particles having a size of 100 to 120 nm. The particles aggregate, and individual particles are seen on the top of the clusters. The particle size is low because of the grounding for more than 12 hours

and the particle aggregation is due to the calcination process. The extremely small size of nanoparticles allows them to penetrate cells and interact with cellular molecules.

Nanoparticles have significant properties that can be used to enhance drug delivery. As the particle is in nano size, a low dose of the drug is enough to treat diseases. Hence *Poonaga Parpam* which is prepared biologically contains nanoparticles to enhance fast pharmacological action in target site.

EDAX analysis shows the elements present in the sample as shown in Fig.9. The table represents the weight and atomic percentage of sample. The quantitative estimation of copper and Sulphur in the test drug *Poonaga Parpam* is 44.62 Wt % and 48.65 Wt% respectively which may be due to the presence of earthworms. The presence of Tc and K is very small which may be contributed by the presence of extract of *aduthinna paalai* in the sample.

5.2.7. (XRD) X-RAY DIFFRACTOMETRY

XRD is an essential tool for analyzing important physical parameters like crystalline structure, grain size, etc.,

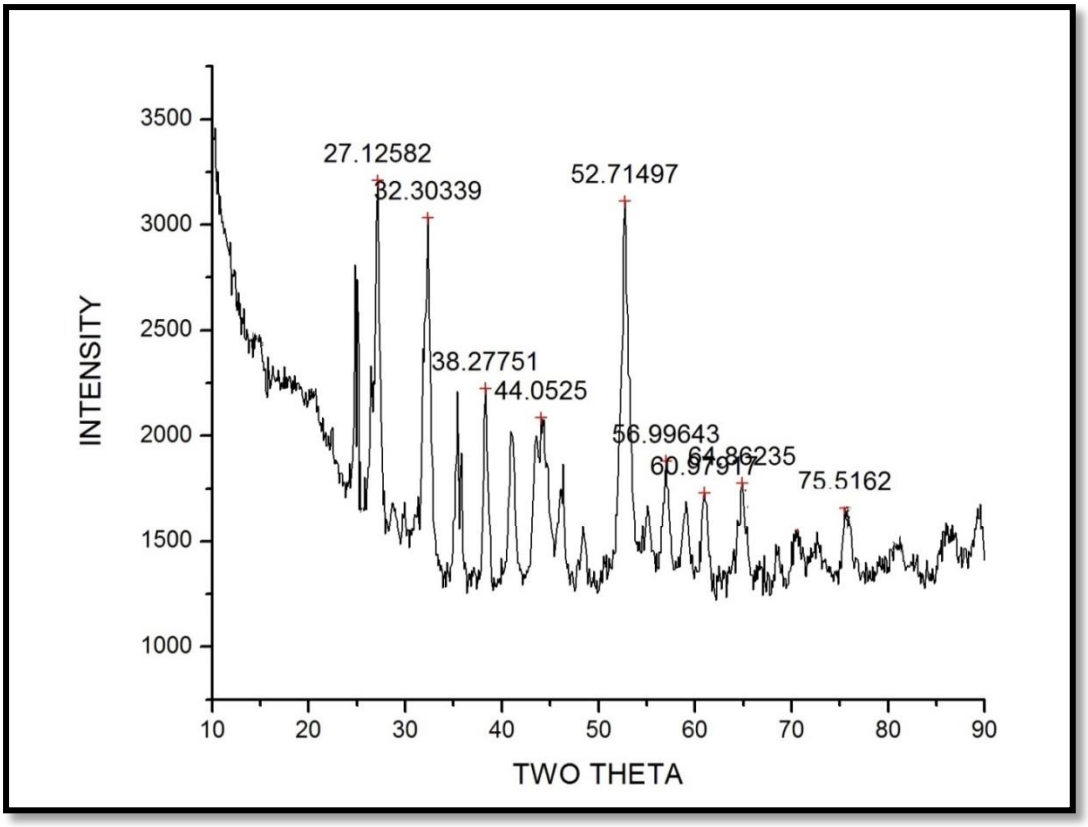


Fig.No.10 XRD image of *Poonaga parpam*

- The X-Ray Diffraction pattern of the Drug *Poonaga Parpam* reveals the presence of major peak with two theta value of 27.12 with the intensity of 3250.
- Major peaks observed in test sample with two theta values of 27.12 and their corresponding intensities matching with the material copper.
- The X-Ray Diffraction pattern of the test drug *Poonaga Parpam* reveals the presence of major peak with two theta value of 32.30 with the intensity of 3100 corresponds to iron.
- Further from this observation it was concluded that copper and iron may the key ingredients present in the test drug *Poonaga Parpam*.

5.2.8. THERMOGRAVIMETRIC ANALYSIS (TGA):

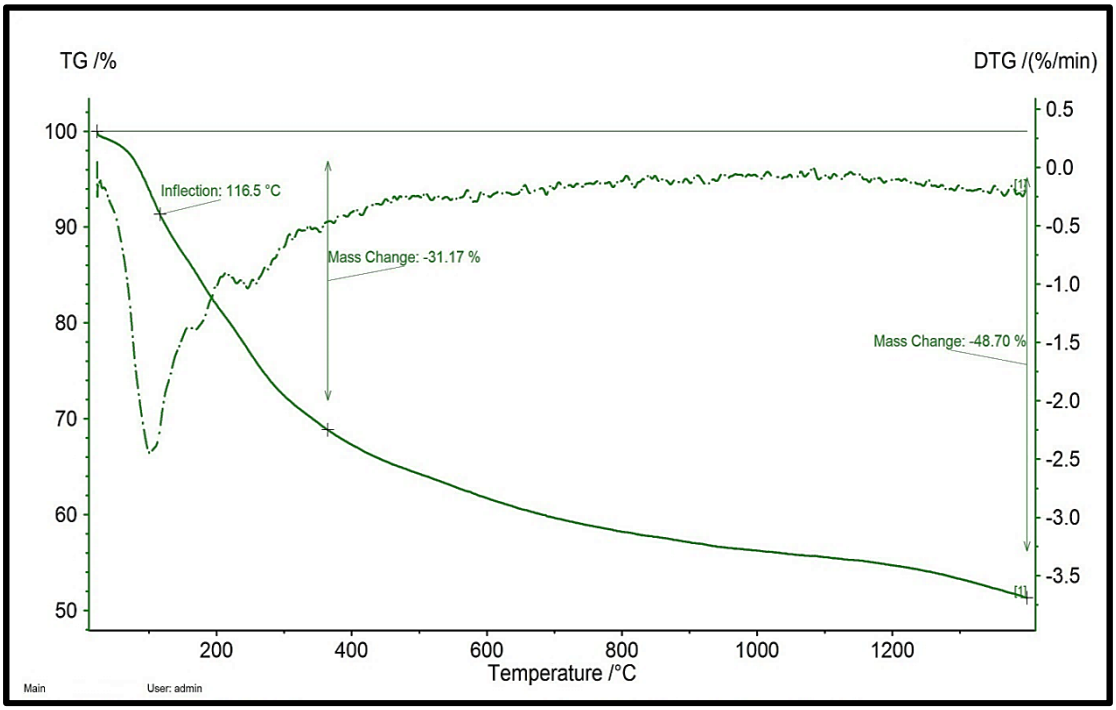


Fig.No.11 Thermal chemical characterization of *Poonaaga parpam*

- Thermo Gravimetric Analysis of *Poonaga Parpam* carried out at the maximum of 1300 degree centigrade. The main objective of the study is to evaluate the decomposition and stability limit of the test drug *Poonaga Parpam.*
- The test drug *Poonaga Parpam* seems to be stable at the temperature varying from 100°C to 380°C.
- Point of decomposition begins when the temperature increases beyond 400°C.
- Weight of the final residual matter was observed with 48.70 % of residual volume.
- From the result of the present investigation it was concluded that the test drug *Poonaga Parpam* seems to be stable at varying temperature ranges from 50 to 400°C.

6. PHARMACOLOGICAL STUDIES

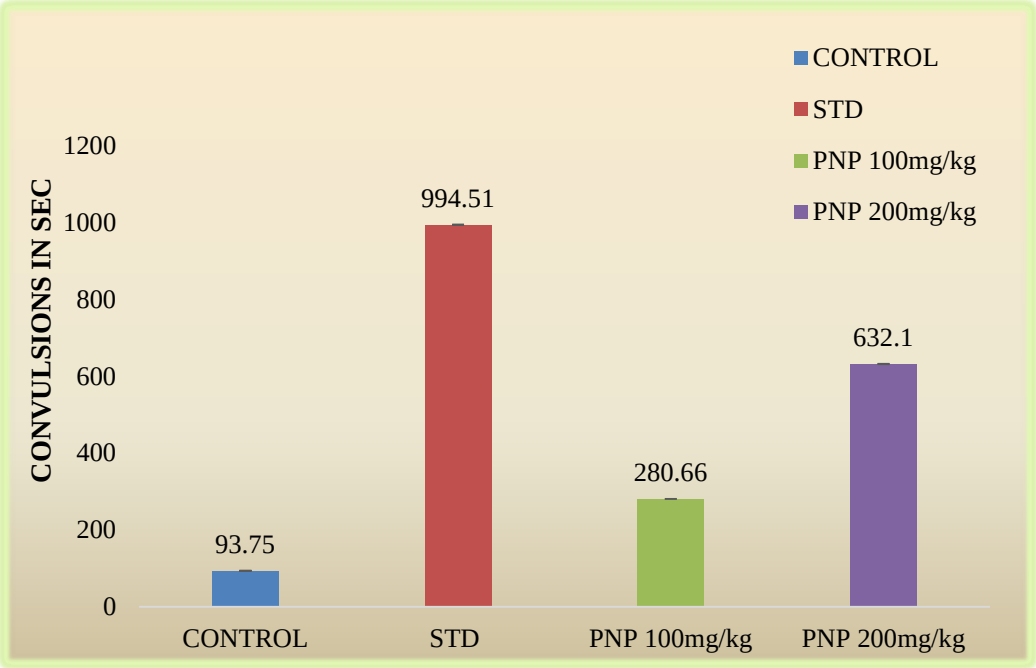
6.1. EVALUATION OF BRONCHODILATOR ACTIVITY OF *POONAGA PARPAM* USING HISTAMINE INDUCED BRONCHO CONSTRICTION IN GUINEA PIG

Table.No.8: Effect of *Poonaga Parpam* against Histamine induced bronchoconstriction in guinea pig

Groups	Onset of Convulsion in sec.	% protection
Group-I **Vehicle Control- Honey (p.o)**	93.75±0.39	--
Group-II **Standard (Chlopheniramine maleate – 2mg/kg, i.p.)**	994.51±0.44**	100
Group-III ***Poonaga parpam*** **(100mg/kg, p.o)**	280.66±.0.22 **	29
Group-IV ***Poonaga parpam*** **(200mg/kg, p.o)**	632.10±0.22**	64

The results were expressed as mean ± SD and was analyzed statistically using one way ANOVA followed by Dunnett's multiple comparisons test.

Chart.No.1: Bronchodilator effect of ***Poonaga Parpam*** in Guinea pig



Histamine induced broncho-constriction is the traditional immunological model of antigen induced airway obstruction. Histamine when inhaled causes hypoxia and leads to convulsion in the guinea pigs and causes very strong smooth muscle contraction, profound hypotension, and capillary dilation in the cardiovascular system. A prominent effect caused by histamine is severe bronchoconstriction in the guinea pigs that causes asphyxia and death. Bronchodilators can delay the occurrence of these symptoms.

Poonaga parpam significantly ($p<0.01$) and dose dependently increased the time of PCT following exposure to histamine aerosols induced bronchospasm in guinea pigs. The percentage protection was found to be 64% in 200 mg/kg of *Poonaga parpam* treated animals, when compared with the untreated control group. The standard group also significantly ($p<0.01$) delayed the onset of pre convulsive dyspnoea time and the percentage protection was found to be 100%, when compared with untreated control group.

The study resulted in deep-rooted the bronchodilator properties of the trial drug *Poonaga parpam* justifying its claiming in the treatment of asthma.

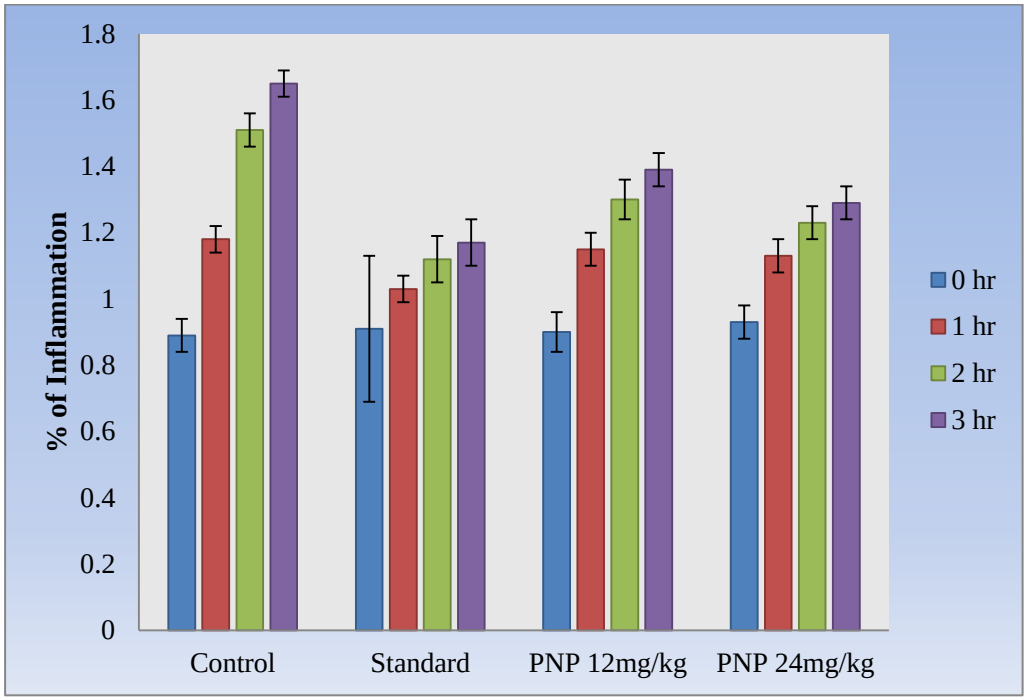
6.2. EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *POONAGA PARPAM* IN WISTAR ALBINO RATS

Table 9 : Inhibitory effect of *Poonaga Parpam* against carrageenan induced paw oedema in Wistar albino rats.

Groups	Percentage of inflammation after carrageenan injection at different hrs			
	0 hr	**1 hr**	**2 hrs**	**3 hrs**
Group-I **Vehicle Control-Honey (p.o)**	0.89±0.05	1.18±0.04	1.51±0.05	1.65±0.04
Group-II **Standard Indomethacin 10 mg/kg(p.o)**	0.91±0.22	1.03±0.04***	1.12±0.07***	1.17±0.07***
Group-III ***Poonaga Parpam* 12 mg/kg(p.o)**	0.90±0.06	1.15±0.05	1.30±0.06***	1.39±0.05***
Group-IV ***Poonaga Parpam* 24 mg/kg(p.o)**	0.93±0.05	1.13±0.05	1.23±0.05***	1.29±0.05***

Values are Mean ± SD; n = 6 animals in each group: * P<0.05, ** P< 0.01, ***P<0.001 is considered significant when compared with control rats and followed by one way ANOVA.

Chart.No.2. Anti inflammatory effect of ***Poonaga Parpam*** in Wistar albino rats



The anti-inflammatory activity was evaluated using carrageenan-induced paw edema models in Wistar albino rats. Oedema formation in the carrageenan-induced paw edema model is a biphasic response. In the early hyperemia, 0-2 hrs after carrageenan injection, there is a release of histamine, serotonin and bradykinin on vascular permeability. The inflammatory edema reached its maximum level at 1 hr and after that, it started declining. The late phase of the inflammatory response has been shown to be due to potentiating effect of bradykinin on mediator release and prostaglandins, producing edema after mobilization of the leukocytes. Nitrous oxide (NO) is a potent vasodilator and is also involved in carrageenan-induced edema, which may be related to its ability to increase vascular permeability and edema through changes in local blood flow.

The effect of *Poonaga parpam* on carrageenan-induced rat paw edema at different hours of study was compared to that of control for the evaluation of anti-inflammatory activity on the basis of percent inhibition of paw edema volume. The Group I is carrageenan induced along with oral administration of vehicle honey which showed an elevated level of paw volume in each hour. At the end of the 3rd hour the paw volume is higher than the Initial Paw Volume. In Group II the Standard Indomethacin is orally received which gives low paw volume in each hour (1st to 3rd

hr). Finally at the end of 3rd hour paw volume shows least value. In Group III & IV the Carrageenan is subcutaneously induced along with the oral administration of *Poonaga parpam* of 12 mg/kg and 24 mg/ kg respectively. The anti-inflammatory activity was found to be dose dependent in carrageenan-induced paw edema model. *Poonaga parpam* has shown significant ($P < 0.001$) inhibition of paw oedema on 3rd hour at the doses of 12 and 24 mg/kg, respectively.

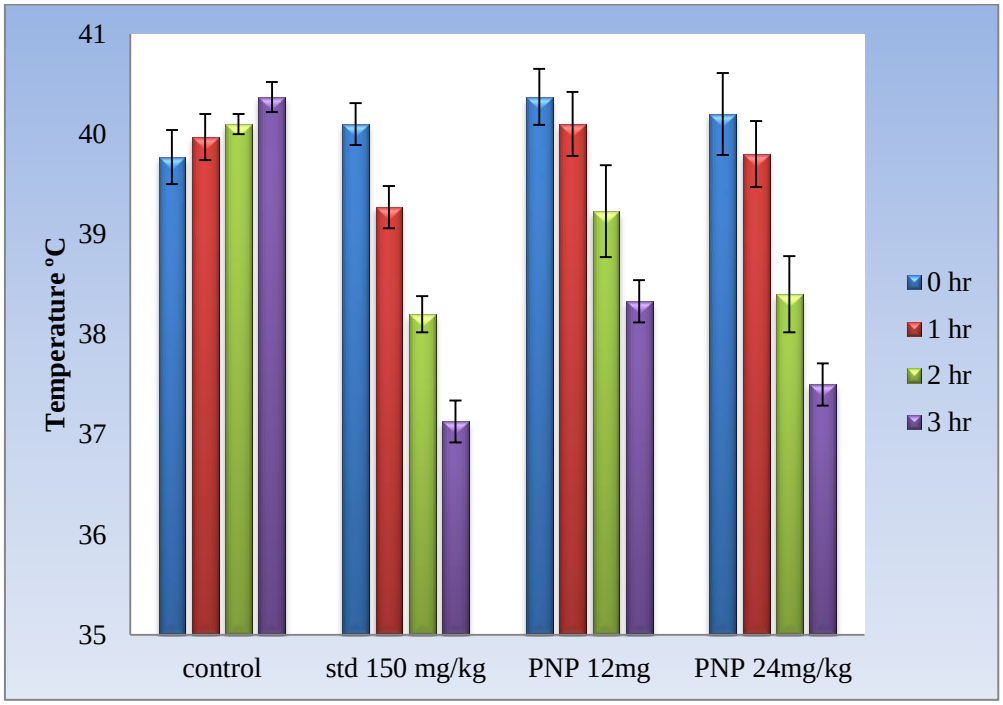
6.3 EVALUATION OF ANTI-PYRETIC ACTIVITY OF *POONAGA PARPAM* IN WISTAR ALBINO RATS

Table. 10 Antipyretic effect of *Poonaga Parpam* in Wistar albino rats by Brewer's yeast induced pyrexia.

Groups	Initial Rectal temp.in °c	Rectal temp.in °c after 8 hrs of yeast injection			
		0hr	**1hrs**	**2hrs**	**3hrs**
GroupI **Control Honey (p.o)**	36.97±0.27	39.77±0.23	39.97±0.23	40.10±0.10	40.37±0.15
Group-II Standard **Paracetamol 150mg/kg p.o**	36.50±0.21	40.10±0.21	39.27±0.24***	38.20±0.18***	37.13±0.21***
Group-III ***Poonaga Parpam*** **12mg/kg p.o**	36.90±0.28	40.37±0.32	40.10±0.30	39.23±0.46***	38.33±0.21***
Group-IV ***Poonaga Parpam*** **24mg/kg p.o**	36.73±0.41	40.20±0.33	39.80±0.40	38.40±0.38***	37.50±0.21***

Values are Mean ± SD; n = 6 animals in each group: * P<0.05, ** P< 0.01, ***P<0.001 is considered significant when compared with control rats and followed by one way ANOVA.

Chart.No.3. Antipyretic effect of ***Poonaga Parpam*** in Wistar albino rats



Antipyretic activity of *Poonaga parpam* were evaluated using Brewer's yeast-induced hyperpyrexia in rats. The subcutaneous injection of yeast suspension markedly elevated the rectal temperature at the 8th hour after administration. The results obtained from the study showed that there was significant increase in the body temperature of rats injected with Brewer's yeast. The antipyretic effect started as early as the first hour after administration, and the effect was maintained for three hours after its administration.

Rats treated with the standard drug Paracetamol (150 mg/kg) has shown maximum reduction in rectal temperature during 3rd hour after injection of Brewer's yeast. It was found that *Poonaga parpam* at doses of 12 mg/kg and 24 mg/kg caused significant lowering of body temperature when compared to the control group animals. Inhibition of prostaglandin synthesis could be the possible mechanism of anti pyretic action as that of paracetamol. Also, there are several mediators or multiprocesses underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about anti pyretic activity.

8. DISCUSSION

The trial drug *Poonaga parpam* was selected from the text "***Sikicha Rathina Deepam****"* for Standardization and screening the pharmacological effect of Bronchodilator, Anti inflammatory and Anti pyretic activities

The drug was prepared as per the procedure and subjected to various studies to reveal its potency and effectiveness against the disease.

Various collections of Siddha and Modern science about the ingredients of the drug supported the fact of Bronchodilator activity. *Poonaga parpam* had been subjected to various studies and it confirms the literature evidences. Literary collections, Chemical analysis, Elemental analysis and Pharmacological studies were done to prove the Bronchodilator, Anti inflammatory and Anti pyretic activities of *Poonaga parpam.*

Literary review about the ingredients of *Poonaga Parpam* from various text books give hope about its activity. The studies strongly substantiated textual references and as discussed below.

Literary collections:

Literary collections include drug review, which consist of Botanical aspect, Zoological aspect, *Gunapadam* aspect, pharmaceutical and pharmacological aspects which support this study.

Drug review:

Botanical aspect:

Drug review about the ingredients of *Poonaga parpam* from various text books were done. Botanical aspect deals with the identification, description, ethno medical important of the plants.

Gunapadam aspect:

Basically siddha medicines have five unique properties. They are

- *Suvai* (Taste)
- *Gunam* (Properties)
- *Veeriyam* (Potency)
- *Pirivu* (Class)
- *Mahimai* (Action)

All the five properties are based on the *Panchabootham* (Five elements) present in the drug. According to our literature, derangement of *Kaba* causes *Kodiya Kaasam*

"கபமல்லாது காச சுவாசம் வாராது"

The therapeutic potency of any drug were designed depending on the following parameters namely

- *Suvai*
- *Gunam*
- *Veeriyam*
- *Vibhaham*

In Siddha system of medicine there is an interrelation between *veeriyam* and treatment. ***Veppa Veeriyam*** normalizes the *Kaba kutram.*

The ingredients of *Poonaga parpam* are *Poonagam* (earthworm) and *Aaduthinnapaalai* (*Aristolochia bracteata*).

- *Poonagam* has *Veppa Veeriyam* and Stimulant action
- *Aaduthinnapaalai has Veppa Veeriyam* and Stimulant action

Majority of the ingredients of *Poonaga parpam* possesses ***Veppa Veeriyam*** and Stimulant action. *Sembu sathu* which is present enormously in *Poonagam* also posseses *Veppa Veeriyam* and Stimulant action. Hence it normalize the *Kaba kutram.* So the selected drug acts as a Bronchodilator.

These collections showed the effectiveness of *Poonaga Parpam* in Bronchial asthma.

Physico-chemical analysis

- The trial drug *Poonaga parpam* showed that the Loss on drying (LOD) was less than 1% which reveals the low moisture content present in the prepared medicine. Low moisture content- drug could get maximum stability and better shelf life.
- The trial drug is found to have total ash value 24.72% , whereas the acid insoluble ash and water soluble ash was 0.72% and 6.94% respectively. The

value of total ash in the formulation is high because of the presence of inorganic ingredients and the method of preparation of this drug is calcination procedure.

- pH of the trial drug was 8.4. It shows the alkalinity of the drug.

Chemical analysis:

Chemical analysis of the drug *Poonaga Parpam* revealed the presence of Silicate, Copper, Sulphate, Carbonate, Iron and Calcium.

Instrumental analysis:

Based on the results, *Poonaga Parpam* is preferably non-toxic to human in its therapeutic dose. The standardization of the drug was evaluated by chemical characterization with heavy metal analysis, functional group analysis, elemental analysis, thermal analysis and determination of particle size by ICP-OES, FTIR, XRD, TGA and SEM EDAX respectively.

- ICP-OES reveals high concentration of Cu in *Poonaga parpam* (21.561 mg/l). It also has physiologically important minerals like Ca, Fe, K, Mg, Na, P and S. In *Poonaga parpam* , the heavy metals like As, Cd, Pb, Hg and trace element like Ni were below detectable level. This reveals the safety of the drug.
- The FTIR results showed the presence of O-H stretch, C-H stretch, C=O stretch, acyclic stretch, -C-H bending, C-N stretch, C-F stretch, C-Cl stretch as functional groups. This indicates the presence of some organic functional groups such as alcohol, aromatic, alkane, carbonyl, ketone, alkane, amine and alkyl halide.
- XRD results revealed that the copper and iron may be the key ingredients present in the test drug *Poonaga Parpam.*
- TGA results revealed that the test drug *Poonaga Parpam* seems to be stable at varying temperature ranges from 50 to 400°C. Point of decomposition begins when the temperature increases beyond 400°C.
- The SEM picture shows that the particles are in nano size and have pores in the drug *Poonaga Parpam.* Further, the study shows that *Poonaga Parpam* is

a kind of nano medicine which favours the advantages of bio availability, better absorption and non toxic with minimal dose level.

The chemical analysis and elemental analysis shows the presence of Cu as a major compound.

PHARMACOLOGICAL STUDIES:

The pharmacological activities like Bronchodilator, Anti inflammatory and Anti pyretic activity of *Poonaga parpam* shows significant effect.

BRONCHODILATOR ACTIVITY

The Bronchodilator activity of the *Poonaga Parpam* has been estimated in the Histamine induced bronchoconstriction in guinea pig. Increase in the Pre convulsive time (PCT) and increase in the percentage protection are the important criteria for the test drug in the management of asthma. *Poonaga Parpam* at the dose of 100 mg/kg, 200 mg/kg significantly and dose dependently increased the time of PCT compared with Chlopheniramine maleate – 2mg/kg treated Group. Experimental groups (III- IV) were compared with control group. Values are statistically significant at $P<0.01$.

ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory activity was evaluated using carrageenan-induced paw edema models in Wistar albino rats. The anti-inflammatory activity was found to be dose dependent in carrageenan-induced paw edema model. *Poonaga Parpam* has shown significant ($P < 0.001$) inhibition of paw oedema on 3^{rd} hour at the doses of 12 and 24 mg/kg, respectively.

ANTI-PYRETIC ACTIVITY

The Anti pyretic activity of the *Poonaga parpam* has been estimated rats by Brewer's yeast induced pyrexia in Wistar albino rats. The result indicates that *Poonaga parpam* at doses of 12 mg/kg and 24 mg/kg caused significant lowering of body temperature when compared to the control group animals. Values are statistically significant at $P<0.001$.

9.SUMMARY

- The test drug *Poonaga Parpam* , a traditional Siddha formulation was selected from the classical siddha literature ***Sikicha Rathina Deepam*** for its Bronchodilator, Anti inflammatory and Anti pyretic activities
- The test drug was prepared as per the Standard operative procedure mentioned in Siddha literature. All the ingredients were identified and authenticated by the experts.
- Review of Literature in various categories was carried out. Siddha aspect, botanical aspect, Zoological aspect, Pharmaceutical and pharmacological review disclosed about the drug and the disease.
- The drug was subjected to analysis such as physicochemical, chemical, instrumental and pharmacological analysis.
- Chemical analysis of the drug *Poonaga Parpam* revealed the presence of Silicate, Copper, Sulphate, Carbonate, Iron, Calcium.
- The presence of organic functional groups such as alcohol, aromatic, alkane, carbonyl, ketone, alkane, amine and alkyl halide were identified in *Poonaga Parpam* by using Fourier Transform Infra-Red Spectroscopy (FTIR).
- The presence of heavy metals in *Poonaga Parpam* were identified within the WHO permissible limits by using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)
- The particle size and Identification and quantitative analysis of chemical elements were assessed by Scanning Electron Microscope with Energy Dispersive X-Ray Analysis (SEM with EDAX). The SEM analysis of *Poonaga Parpam* reveals that the majority of the particles are nano in size.
- Characterization and Identification of crystalline materials were assessed by X-Ray Diffractometer (XRD). The particles of *Poonaga Parpam* shows some crystallinity in nature.
- The thermal analysis of *Poonaga Parpam* by Thermogravimetric analysis or thermal gravimetric analysis (TGA). Point of decomposition begins in the sample *Poonaga Parpam* when the temperature increases beyond 400°C.

- Pharmacological studies were done. It revealed that the drug *Poonaga Parpam* possess Bronchodilator, Anti-inflammatory and Anti pyretic activities in animal model.
- From the results and statistical analysis it was proved that the drug *Poonaga Parpam* has
    1. Bronchodilator activity
    2. Anti-inflammatory activity
    3. Anti pyretic activity

- This present study suggests *Poonaga Parpam* has remarkable medicinal value in the treatment of Bronchial Asthma. Thus the Siddha formulation *Poonaga Parpam* is Standardized and validated for its efficacy in treating Bronchial asthma (*Kaasam*) and it would be a great drug of choice.

10. CONCLUSION

From the Literature evidence, Physico chemical analysis, chemical analysis, Elemental analysis and Pharmacological studies, the drug *Poonaga Parpam* have potent Bronchodilator, Anti-inflammatory and Anti pyretic activity. It was concluded that the *Poonaga Parpam* can be used in the management of *Kaasam* (Bronchial asthma).

Future scope

The pharmacological activities carried out in animals for Bronchodilator activity supported the study. But to evaluate the drug *Poonaga Parpam* in a better way, clinical research is required so that the exact benefit and aim of the study would be fulfilled and a good drug to fill the space for an effective anti asthmatic drug would be achieved. Although SEM depicted the presence of nano particles, the active compound exhibiting the therapeutic efficacy should be analysed. If these lacunas are corrected the *Poonaga Parpam* would be proficient with the new hope in the treatment of Bronchial asthma.

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Workshop on

"BASIC RESEARCH TECHNIQUES AND PRACTICES INVOLVED IN LABORATORY ANIMAL CARE"

06 -10 February 2017

CERTIFICATE

This is to certify that Dr. V.Elakkiya has participated as Delegate/~~Resource~~ Person in the workshop on "Basic Research Techniques and Practices involved in Laboratory Animal Care" held on 06-10 February, 2017 at National Institute of Siddha, Chennai-47, Tamilnadu.

Dr. V. Suba
Organizing Secretary

Dr. P. Muthusamy
Veterinary Consultant

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The Tamil Nadu Dr. M.G.R. Medical University From 25th to 29th April 2016.

Dr.N.KABILAN, MD(S),
PROF & HEAD
DEPT.OF SIDDHA

Prof.**Dr.P.ARUMUGAM,** M.D.,
REGISTRAR i/c

Prof. **Dr.S.GEETHALAKSHMI,** M.D., Ph.D.,
VICE CHANCELLOR

CERTIFICATE

This is certify that the project title **Standardization and Pharmacological screening of "*PoonagaParpam (PNP)*"** has been approved by the IAEC. 24 Rats (12M+12F)
Approval No: NIS/IAEC-III/03/29092016

Prof. Dr. V. Banumathi — Prof. Dr. K. Nachimuthu

Name of Chairman/~~Member Secretary~~ IAEC: **Name of CPCSEA nominee:**

Signature with date

V. Banumathi 17/11/16

Chairman/~~Member Secretary~~ of IAEC: **CPCSEA nominee:**

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

Name of the PI: ~~Dr. T. Ethaya~~ Dr. V. Ekakkiya

Name of the Dept: Gunapadam - Branch II

C.L.BAID METHA COLLEGE OF PHARMACY

(An ISO 9001-2000 certified institute)

Jyothi Nagar, Old Mahabalipuram Road

Thoraipakkam, Chennai – 600 097

CERTIFICATE

This is to certify that the project entitled, Pharmacological screening of Poonaga Parpam submitted in partial fulfilment for the degree of M.D. (siddha) was carried out at C.L. Baid Metha college of Pharmacy, Chennai-97, in the Department of Pharmacology during the academic year of 2017-2018. It has been approved by the IAEC No: LI/23/CLBMCP/2017

C.L. Baid Metha College of Pharmacy ★ CHENNAI-97 ★ Thoraipakkam

Dr.P.MURALIDHARAN

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drug used in the Siddha formulation **Poonaga parpam** (Internal) taken up for Post Graduation Dissertation studies by **Dr.V.Elakkiyaa** M.D.(S), II year, Department of Gunapadam, 2017, is identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology, Micromorphology and Taxonomical methods as

Aristolochia bracteata Retz. (Aristolochiaceae), Whole plant

MEDICINAL BOTANY CHENNAI 600 047 National Institute of Siddha

Certificate No: NISMB2742017

Date: 13-02-2017

Authorized Signatory

Dr. D. ARAVIND, M.D.(s),M.Sc.,
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15.02.17

AUTHENTIFICATION CERTIFICATE

Certified that the sample submitted for identification by **Dr. V. Elakkiyaa**, II year PG scholar, Dept. of Gunapadam, National Institute of Siddha, Chennai - 47, is identified as **Earth worm- Lumbricus terrestris L.** on the basis of macroscopic character.

This certificate is issued for the purpose of preparing her dissertation medicine in Gunapadam laboratory, NIS.

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